

(19) World Intellectual Property
Organization
International Bureau



(43) International Publication Date
17 February 2005 (17.02.2005)

PCT

(10) International Publication Number
WO 2005/013666 A2

(51) International Patent Classification: **Not classified**

(21) International Application Number:
PCT/AU2004/001057

(22) International Filing Date: 9 August 2004 (09.08.2004)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
2003904237 8 August 2003 (08.08.2003) AU

(71) Applicant (for all designated States except US): **GARVAN INSTITUTE OF MEDICAL RESEARCH** [AU/AU]; St Vincent's Hospital, 384 Victoria Street, Darlinghurst, New South Wales 2010 (AU).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **JAMES, David**

[AU/AU]; 25 Cutler Road, Clontarf, New South Wales 2093 (AU). **GOVERS, Roland** [NL/NL]; Vioolstraat 25, NL-4702 CK Roosendaal (NL).

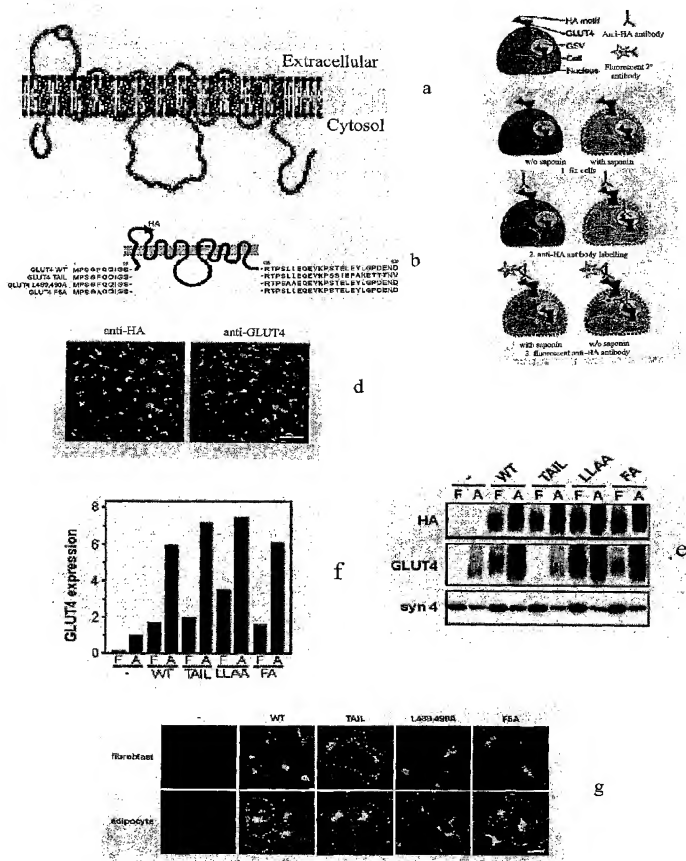
(74) Agent: **F. B. RICE & CO**; 139 Rathdowne Street, Carlton South, Victoria 3053 (AU).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM,

[Continued on next page]

(54) Title: NOVEL TRANSLOCATION ASSAY



(57) Abstract: The present invention relates to a novel in vitro assay for determining the level of a protein, in particular, a membrane transport protein that is located at the plasma membrane of a cell compared to the level of the protein in the cell. The process of the invention is also useful for determining the level of recycling of a membrane transport protein. The present invention additionally provides a process for identifying an agent that modulates the translocation of a protein, in particular, a membrane transport protein, to the plasma membrane and, as a consequence, the activity of that protein.



ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

Published:

- *without international search report and to be republished upon receipt of that report*

Novel translocation assay

Field of the invention

The present invention relates to a novel *in vitro* assay for determining the level of a protein, in particular, a membrane transport protein that is located at the plasma membrane of a cell compared to the level of the protein in the cell. In one embodiment, the present invention provides a method for identifying an agent that modulates the translocation of a protein, in particular, a membrane transport protein, to the plasma membrane and, as a consequence, the activity of that protein.

Background of the Invention*General*

This specification contains nucleotide and amino acid sequence information prepared using PatentIn Version 3.1, presented herein after the claims. Each nucleotide sequence is identified in the sequence listing by the numeric indicator <210> followed by the sequence identifier (e.g. <210>1, <210>2, <210>3, etc). The length and type of sequence (DNA, protein (PRT), etc), and source organism for each nucleotide sequence, are indicated by information provided in the numeric indicator fields <211>, <212> and <213>, respectively. Nucleotide sequences referred to in the specification are defined by the term "SEQ ID NO:", followed by the sequence identifier (eg. SEQ ID NO: 1 refers to the sequence in the sequence listing designated as <400>1).

The designation of nucleotide residues referred to herein are those recommended by the IUPAC-IUB Biochemical Nomenclature Commission, wherein A represents Adenine, C represents Cytosine, G represents Guanine, T represents thymine, Y represents a pyrimidine residue, R represents a purine residue, M represents Adenine or Cytosine, K represents Guanine or Thymine, S represents Guanine or Cytosine, W represents Adenine or Thymine, H represents a nucleotide other than Guanine, B represents a nucleotide other than Adenine, V represents a nucleotide other than Thymine, D represents a nucleotide other than Cytosine and N represents any nucleotide residue.

As used herein the term "derived from" shall be taken to indicate that a specified integer may be obtained from a particular source albeit not necessarily directly from that source.

Throughout this specification, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated step or element or integer or group of steps or elements or integers but not the exclusion of any other step or element or integer or group of elements or integers.

Throughout this specification, unless specifically stated otherwise or the context requires otherwise, reference to a single step, composition of matter, group of steps or group of compositions of matter shall be taken to encompass one and a plurality (i.e. one or more) of those steps, compositions of matter, groups of steps or group of compositions of matter.

Each embodiment described herein is to be applied *mutatis mutandis* to each and every other embodiment unless specifically stated otherwise.

15

Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations or any two or more of said steps or features.

The present invention is not to be limited in scope by the specific embodiments described herein, which are intended for the purpose of exemplification only. Functionally-equivalent products, compositions and methods are clearly within the scope of the invention, as described herein.

The present invention is performed without undue experimentation using, unless otherwise indicated, conventional techniques of molecular biology, microbiology, virology, recombinant DNA technology, peptide synthesis in solution, solid phase peptide synthesis, and immunology. Such procedures are described, for example, in the following texts that are incorporated by reference:

Sambrook, Fritsch & Maniatis, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratories, New York, Second Edition (1989), whole of Vols I, II, and III;
DNA Cloning: A Practical Approach, Vols. I and II (D. N. Glover, ed., 1985), IRL Press, Oxford, whole of text;

- Oligonucleotide Synthesis: A Practical Approach (M. J. Gait, ed., 1984) IRL Press, Oxford, whole of text, and particularly the papers therein by Gait, pp1-22; Atkinson *et al.*, pp35-81; Sproat *et al.*, pp 83-115; and Wu *et al.*, pp 135-151;
- Nucleic Acid Hybridization: A Practical Approach (B. D. Hames & S. J. Higgins, eds., 5 1985) IRL Press, Oxford, whole of text;
- Animal Cell Culture: Practical Approach, Third Edition (John R.W. Masters, ed., 2000), ISBN 0199637970, whole of text;
- Immobilized Cells and Enzymes: A Practical Approach (1986) IRL Press, Oxford, whole of text;
- 10 Perbal, B., A Practical Guide to Molecular Cloning (1984);
- Methods In Enzymology (S. Colowick and N. Kaplan, eds., Academic Press, Inc.), whole of series;
- J.F. Ramalho Ortigão, "The Chemistry of Peptide Synthesis" *In*: Knowledge database of Access to Virtual Laboratory website (Interactiva, Germany);
- 15 Sakakibara, D., Teichman, J., Lien, E. and Fenichel, R.L. (1976). *Biochem. Biophys. Res. Commun.* **73** 336-342
- Merrifield, R.B. (1963). *J. Am. Chem. Soc.* **85**, 2149-2154.
- Barany, G. and Merrifield, R.B. (1979) in *The Peptides* (Gross, E. and Meienhofer, J. eds.), vol. 2, pp. 1-284, Academic Press, New York.
- 20 Wünsch, E., ed. (1974) *Synthese von Peptiden in Houben-Weyls Methoden der Organischen Chemie* (Müller, E., ed.), vol. 15, 4th edn., Parts 1 and 2, Thieme, Stuttgart.
- Bodanszky, M. (1984) *Principles of Peptide Synthesis*, Springer-Verlag, Heidelberg.
- Bodanszky, M. & Bodanszky, A. (1984) *The Practice of Peptide Synthesis*, Springer- 25 Verlag, Heidelberg.
- Bodanszky, M. (1985) *Int. J. Peptide Protein Res.* **25**, 449-474.
- Handbook of Experimental Immunology, Vols. I-IV (D. M. Weir and C. C. Blackwell, eds., 1986, Blackwell Scientific Publications).
- 30 *Description of the related art*
- An important activity performed by any cell is the transport of materials across the plasma membrane. This activity is essential for the survival of all organisms, from simple unicellular organisms, e.g. bacteria, to complex multicellular organisms, e.g. humans. Not only does membrane transport facilitate the uptake of, for example,
- 35 nutrients and ions, but also the excretion of waste products, and the secretion of signaling molecules..

The process of membrane transport itself is performed by a large class of proteins known as “transporters” “membrane transporters” “membrane transport proteins”. A number of these proteins function by forming protein channels in the plasma membrane of a cell. This class of proteins includes a vast number of proteins that are related by their ability to transport other molecules across a cell membrane. It is hypothesized that the number of proteins involved in membrane transport constitute approximately 5% to 10% of known open reading frames in most sequenced genomes.

- 10 Membrane transport proteins are generally localized both intracellularly and within the plasma membrane. However, as the membrane-localized form is capable of transport activity, the amount of any membrane transport protein present in the plasma membrane limits the transport of substrates (both naturally-occurring substrates and small molecules) into and/or outside of the cell. Exemplary membrane transport
- 15 proteins include the glucose-transporters (e.g. GLUT1, GLUT4), water transporters (e.g., aquaporins) and ion transporters that transport Cl^- , K^+ , Na^+ , Cu^{2+} or SO_4^{2-} ions, amongst others (e.g. cystic fibrosis transmembrane regulator (CFTR), pendrin, human ether-a-go-go (HERG)). As will be known to those skilled in the art, membrane transport proteins may function in the transport of multiple substrates for example, in
- 20 the same direction (e.g., symport) across the plasma membrane or in the opposite direction (eg., antiport) across the plasma membrane.

Cells utilize a number of transport mechanisms, all of which are controlled by transport proteins.

25

Facilitated diffusion utilizes membrane protein channels to allow charged molecules (which otherwise could not diffuse across a plasma membrane) to freely move across a plasma membrane. For example, K^+ , Na^+ , and Cl^- are transported across a plasma membrane by such membrane protein channels.

30

Facilitative transport molecules convey molecules, such as, for example, sugars down a concentration gradient, i.e. from a region of high concentration of that molecule to a region of low concentration, in a process that does not require energy.

In contrast, active transport requires the expenditure of energy to transport the molecule across the membrane. Similar to facilitated transport, active transport is limited by the number of membrane transport proteins present at the membrane.

- 5 Active, or coupled, membrane transporters transport substrates against a concentration gradient in a process that either requires energy expenditure or the use of another concentration gradient. For example, sodium dependent glucose transporters couple the transport of one molecule of glucose to two molecules of sodium. Sodium ions are transported down their concentration in a process that generates sufficient free energy
10 to transport glucose against its concentration gradient allowing for a significant increase in the concentration of glucose in a cell.

As membrane transport proteins are involved in such a variety of functions that are essential to the survival of an organism, it is not surprising that several of these proteins
15 have been found to be associated with disease in humans. For example, several forms of hearing loss in humans are associated with mutations in genes encoding transport proteins such as, for example, connexin 26, and pendrin, a proposed sulfate transporter. Defects in ion transporters are associated with a predisposition to cardiac arrhythmia, Menke's disease, Wilson's disease, familial generalized epilepsy, benign infantile
20 epilepsy, spinocerebellar ataxia and familial hemiplegic migraine amongst many others.

Additionally, deficiency of the water channel protein aquaporin 2 hinders its translocation to the apical surface of the cell abolishing reabsorption of water from the collecting duct and resulting in nephrogenic diabetes insipidus.

25

Diabetes is associated with a dysfunctional glucose uptake into muscle and fat cells due to the impaired ability of insulin to stimulate glucose transporters.

In addition to mutations that directly affect the activity of a protein, any defect that
30 inhibits the trafficking of the relevant membrane transport protein to the correct subcellular location has also been shown to be linked with human disease. For example, it has been suggested that the membrane transport protein GLUT4 is abnormally localized in type II diabetes (Bryant *et al*, *Nature Reviews Molecular Cell Biology*, 3, 267-277, 2002). In a normal cell GLUT4, which transports glucose across
35 the plasma membrane, is thought to be almost entirely intracellular in the absence of insulin. Upon the addition of insulin, GLUT4 translocates to the plasma membrane.

However, in skeletal muscle cells from some type II diabetes mellitus subjects (Kelley *et al*, *J. Clin. Invest.* 97, 2705-2713, 1996) GLUT4 translocation has been shown to be drastically reduced. These results suggest impaired glucose transport as a consequence of impaired GLUT4 translocation may play a role in insulin resistance in type II
5 diabetes.

The most common mutations in the cystic fibrosis transmembrane regulator (CFTR) gene (the Δ F508 mutation, Δ 1507 mutation, K464M mutation, F508R mutation, and S5491 mutation, which account for approximately 70% of CF patients) have been
10 suggested to cause abnormal localization of the CFTR protein to the endoplasmic reticulum, where it is subsequently degraded (Cheng *et al*, *Cell*, 63(4), 827-834, 1990). Such mutant forms of the CFTR protein have been observed to be localized at the apical region of the cytosol of cells, rather than within the plasma membrane. As the CFTR protein is a chloride channel, the reduction in the amount of this channel in the
15 membrane is associated with reduced movement of both sodium and water into the cell. The mislocalization of the CFTR protein has also been suggested as a possible causative factor in the reduced movement of sodium and water observed in the lungs and intestines of subjects suffering from cystic fibrosis.

20 In the case of cardiac arrhythmia, mutations have been found in the genes encoding the potassium channels, human ether-a-go-go-related gene (HERG), and KVLQT1. The HERG protein is the pore-forming subunit of the cardiac rapidly activating delayed rectifier potassium channel. In both cases, mutations in the gene encoding each protein are associated with a reduction with trafficking of the protein and, as a consequence, a
25 reduction in the amount of the protein being integrated into the plasma membrane. As a result, cardiac cells expressing the mutant protein show reduced amplitude and altered voltage dependence of activation (Zhou *et al*, *J. Biol. Chem.*, 274(44), 31123-31126, 1999).

30 Mutations in various other membrane transport proteins have also been suggested to cause a number of disorders due to altered or incorrect trafficking/translocation of the mutant protein, for example, glucose-galactose malabsorption, changes in cholesterol homeostasis, and defects in the multi-drug transporter P-glycoprotein.

35 As membrane transport proteins are involved in several essential cellular processes, and mutations affecting the function and/or localization of these proteins are involved in the

etiology of certain human diseases, there is a clear need in the art for methods of detecting mutations in these proteins and/or modulatory agents that affect their subcellular localization and/or turnover/recycling.

- 5 Known methods of determining the activity of a membrane transport protein generally involve the mere measurement of the movement of a specific substrate across a lipid bilayer, such as that found at the membrane of a cell. These methods are imprecise, as any redundancy in the transport process of interest, e.g. if a cell expresses multiple proteins that transport the same molecule, may mask or reduce the effect of a mutation
10 of one of the constituents (i.e. transport proteins) of the process. For example, there are at least 12 hexose transporters encoded by the genes in the human genome and most mammalian cell types express more than one member of this family.

- Alternatively, plasma membranes are isolated and low density microsomal fractions
15 prepared. The membrane transport proteins are then photolabeled (e.g. bis-mannose photolabeling of GLUT4 located on the cell surface), and subsequently immunoprecipitated e.g. as described in Homan *et al.*, *J. Biol. Chem.* 26:5 18172-18179 (1990).

- 20 Alternatively, plasma membrane sheets are prepared for use in microscopic analysis essentially as described in Cushman and Wardzala., *J Biol Chem.* 255:4758-4762 (1980), or by isolation of plasma membrane sheets or lawns for use in microscopic analysis as described in Robinson, *et al.*, *J Cell Biol.* 117:1181-1196 (1992).

- 25 These assays are both laborious and subject to inter-assay variability, and furthermore, are only semi-quantitative. Accordingly, the quantitative nature of these assays is limited. Furthermore, these assays are not readily adapted to high-throughput analysis, for example, for screening compounds that modulate translocation of a membrane transport protein.

- 30 Accordingly, there is a clear need in the art for a straightforward, reproducible method for the detection and estimation of the level of a membrane transport protein translocated to the plasma membrane. Preferred assays will not require sub-cellular fractionation or multiple labeling. Preferred assays will also be useful for determining
35 mutations and/or agents that affect translocation of the membrane transport protein, for example, in a high-throughput assay.

Summary of the Invention

In work leading up to the present invention, the inventors sought to develop an assay that detects the level of a membrane transport protein incorporated into the plasma membrane of a cell compared to the total level of said membrane transport protein within the cell. Furthermore, the inventors sought to use this assay to determine the level of trafficking and/or turnover of the membrane transport protein at the plasma membrane.

For example, the present inventors have developed an assay useful for determining the level of GLUT4 translocation in a cell. The assay uses a GLUT4 protein that is labeled with a tag or marker that facilitates detection of the GLUT4. Preferably, the tag or marker is located within an extracellular domain of the GLUT4 protein. The location of the tag or marker facilitates detection of the GLUT4 protein at the plasma membrane of an intact cell. By determining the level of tagged/marked GLUT4 protein at the plasma membrane of a cell relative to the level of tagged/marked GLUT4 in the cell, the level of GLUT4 translocation is determined.

The present inventors have additionally shown that the process of the present invention is amenable to performance in 96-well and 384-well formats. Accordingly, this assay provides a high throughput screen to determine a modulator of translocation of a membrane transport protein. Such a modulator represents a candidate therapeutic for the treatment of a disease associated with translocation (e.g. aberrant translocation) of a membrane transport protein.

Furthermore, the present inventors have developed a model of insulin resistance observed in subjects suffering from type-II diabetes. This assay provides the basis for a screen to determine a candidate compound for the treatment of insulin resistance e.g. that associated with type-II diabetes.

The present invention provides a process for determining the level of a membrane transport protein translocated to the plasma membrane of a cell, said method comprising:

- (a) determining the level of a membrane transport protein at the plasma membrane of the cell using a method comprising:

- (i) contacting the cell with a ligand that binds to an extracellular domain of the membrane transport protein for a time and under conditions sufficient for the ligand to bind to the membrane transport protein at the plasma membrane of the cell; and
- 5 (ii) determining the level of ligand bound to the membrane transport protein;
- (b) (i) permeabilizing or disrupting the plasma membrane of a cell and contacting the membrane transport protein within the cell with the ligand for a time and under conditions sufficient for the ligand to bind to the
- 10 membrane transport protein; and
- (ii) determining the level of ligand bound to the membrane transport protein; and
- (c) comparing the level of ligand determined at (a) (ii) and (b) (ii) to determine the level of the membrane transport protein at the plasma membrane relative to
- 15 the level of the membrane transport protein inside the cell.

For example, the membrane transport protein is a glucose transport (GLUT) protein.

In an example, the membrane transport protein is GLUT4, e.g., the GLUT4 comprises

20 an amino acid sequence at least 80% identical to the amino acid sequence set forth in SEQ ID NO: 2.

In another example, the membrane transport protein is GLUT1 e.g., the GLUT1 comprises an amino acid sequence at least 80% identical to the amino acid sequence set

25 forth in SEQ ID NO: 12.

In yet another example, the membrane transport protein is a mutant membrane transport protein having a reduced rate of recycling or transporter internalization compared to a wild-type form of the membrane transport protein.

30 For example, the mutant membrane transport protein is a mutant glucose transport (GLUT) protein having a reduced rate of recycling or transporter internalization compared to a wild-type form of the membrane transport protein.

35 For instance, the reduced rate of recycling or transporter internalization of the mutant membrane transport protein increases the level of the mutant membrane transport

protein at the plasma membrane of a cell compared to the level of a wild-type form of the membrane transport protein.

In an example, the mutant GLUT protein is a mutant GLUT4 protein, e.g., the mutant
5 GLUT4 protein comprises an amino acid sequence at least 80% identical to an amino acid sequence selected from the group consisting of SEQ ID NO: 6, SEQ ID NO: 8 and SEQ ID NO: 10.

For example, the membrane transport protein is labeled to facilitate binding of the
10 ligand to the membrane transport protein.

In an example, the label comprises one or more copies of a peptide, polypeptide or protein that is heterologous to the membrane transport protein. For example, the label comprises one or more copies of a peptide, polypeptide or protein selected from the
15 group consisting of influenza virus hemagglutinin (HA) (SEQ ID NO: 15), Simian Virus 5 (V5) (SEQ ID NO: 16), polyhistidine (SEQ ID NO: 17), c-myc (SEQ ID NO: 18), FLAG (SEQ ID NO: 19), GST (SEQ ID NO: 22), MBP (SEQ ID NO: 23), GAL4 (SEQ ID NO: 24), β -galactosidase (SEQ ID NO: 25), enhanced green fluorescence protein (eGFP) (SEQ ID NO: 26), yellow fluorescent protein (SEQ ID NO: 27), soluble
20 modified blue fluorescent protein (SEQ ID NO: 28), soluble-modified red-shifted green fluorescent protein (SEQ ID NO: 29), cyan fluorescent protein (SEQ ID NO: 30), biotin, streptavidin, a peptide comprising the amino acid sequence set forth in SEQ ID NO: 20, a peptide comprising the amino acid sequence set forth in SEQ ID NO: 21, a peptide comprising the amino acid sequence set forth in SEQ ID NO: 31 and mixtures
25 thereof.

In one exemplified form of the invention, the label comprises the amino acid sequence set forth in SEQ ID NO: 8.

30 For example, the label is positioned within an extracellular domain of the membrane transport protein, e.g., the label is positioned within the first extracellular domain of a GLUT protein or a mutant thereof.

For example, the labeled membrane transport protein is a GLUT4 protein or a mutant
35 GLUT4 protein that comprises an amino acid sequence at least 80% identical to an

amino acid sequence selected from the group consisting of SEQ ID NO 4, SEQ ID NO: 6, SEQ ID NO: 8 and SEQ ID NO: 10.

5 In another example, the labeled membrane transport protein is a GLUT1 protein that comprises an amino acid sequence at least 80% identical to the amino acid sequence set forth in SEQ ID NO: 13.

10 In an example of the invention, the cell is a eukaryotic cell, for example, the cell is a mammalian cell, e.g., a cell selected from the group consisting of a 3T3-L1 fibroblast cell, a 3T3-L1 adipocyte cell and a C2C12 cell.

In an example, the ligand capable of binding to the membrane transport protein is an antibody. For example, the antibody is a monoclonal antibody, e.g., an anti-HA tag antibody.

15

For example, the antibody is labeled with a detectable marker selected from the group consisting of an enzyme label, a radiolabel and a fluorescent label, e.g., the antibody is labeled with a fluorescent label.

20 In an example, the plasma membrane is permeabilized or disrupted by contacting the plasma membrane with an agent that permeabilizes or disrupts a membrane for a time and under conditions sufficient for permeabilization or disruption to occur. For example, the agent that permeabilizes or disrupts a membrane is selected from the group consisting of saponin, n-octyl-glucopyranoside, n-Dodecyl β -D-maltoside, N-
25 Dodecanoyl-N-methylglycine sodium salt, hexadecyltrimethylammonium bromide, deoxycholate, a non-ionic detergent, streptolysin-O (SEQ ID NO: 32), α -hemolysin (SEQ ID NO: 33), tetanolysin (SEQ ID NO: 34) and mixtures thereof, e.g., the agent that permeabilizes or disrupts the membrane is saponin.

30 In an example of the invention, the level of the ligand bound to the membrane transport protein is determined by a process comprising contacting the ligand with an antibody that specifically binds to the ligand for a time and under conditions sufficient for an antibody-antigen complex to form and determining the level of the complex wherein the level of the complex indicates the level of the ligand bound to the membrane
35 transport protein.

For example, the level of the ligand bound to the membrane transport protein is determined using an assay selected from the group consisting of immunofluorescence, immunohistochemistry, and an immunosorbent assay, e.g., the level of the ligand bound to the membrane transport protein is determined using a fluorescence linked
5 immunosorbent assay.

In one example, the process of the invention additionally comprises providing the cell expressing the membrane transport protein. For example, providing the cell expressing the membrane protein comprises transforming or transfecting the cell with an
10 expression construct that encodes the membrane protein.

In an example, the process additionally comprises fixing the cell. For example, the cell is fixed prior to or at the same time as permeabilizing or disrupting the plasma membrane of the cell.
15

In an example, the cell is fixed with a compound selected from the group consisting of formaldehyde, paraformaldehyde, alcohol, methanol and glutaraldehyde, e.g., the cell is fixed with formaldehyde.

20 In another example, the present invention additionally comprises inducing translocation of the membrane transport protein to the plasma membrane. For example, inducing translocation of the membrane transport protein to the plasma membrane comprises contacting the cell with an amount of one or more peptides, polypeptides, proteins or compounds sufficient to induce translocation of the membrane transport protein for a
25 time and under conditions sufficient for translocation to occur.

For instance the cell is contacted with sucrose and/or insulin, e.g., the cell is contacted with sucrose and/or insulin in the presence of serum.

30 In another example, the process additionally comprises inducing resistance to translocation of the membrane transport protein in the cell. For example, the membrane transport is a GLUT protein or a mutant GLUT protein and wherein inducing resistance to translocation of the membrane transport protein in the cell comprises contacting the cell with an amount of insulin sufficient to induce resistance
35 to insulin induced translocation for a time and under conditions sufficient for resistance to insulin induced translocation to occur.

For example, the cell is contacted with insulin in the absence of serum, e.g., the cell is contacted with insulin for between about 24 hours and about 48 hours.

- 5 The present invention provides a process for determining the level of a membrane transport protein translocated to the plasma membrane of a cell, said process comprising:
- (a) determining the level of the membrane transport protein at the plasma membrane of a cell using a method comprising:
- 10 (i) contacting a cell with a ligand that binds to an extracellular domain of the membrane transport protein for a time and under conditions sufficient for the ligand to bind to the membrane transport protein; and
- (ii) determining the level of ligand bound to the membrane transport protein;
- 15 (b) determining the level of the membrane transport protein within another cell using a method comprising:
- (i) permeabilizing or disrupting the other cell;
- (ii) contacting the membrane transport protein within the cell with the ligand for a time and under conditions sufficient for the ligand to bind the membrane transport protein;
- 20 (iii) determining the level of ligand bound to the membrane transport protein; and
- (c) comparing the level of ligand detected at (a) (ii) and (b) (iii) to determine the level of the labeled membrane transport protein at the plasma membrane relative to the total level of labeled membrane transport protein.
- 25

For example, the cells are isogenic or from the same cell line.

- 30 For instance, the cells are cultured under substantially similar conditions.

In an example, the level of the membrane transport protein at the plasma membrane of the cell and the level of membrane transport protein within the cell are each determined in a plurality of cells.

For example, the process of the invention additionally comprises normalizing the determined level of ligand bound to the membrane transport protein with regard to the number of cells in which the level of ligand bound to the membrane transport protein is determined.

5

For example, the number of cells is determined by a method comprising contacting the cells with an antibody or ligand capable of binding to a cell or component thereof for a time and under conditions sufficient for binding of the antibody or ligand to the cell or component thereof and determining the level of antibody bound to the cells, wherein
10 the level of antibody or ligand bound to the cells is indicative of the number of cells, e.g., the ligand is wheat germ agglutinin.

The present invention additionally provides a process for determining the level of a labeled GLUT4 protein or labeled mutant GLUT4 protein translocated to the plasma
15 membrane of a cell, wherein said labeled mutant GLUT4 protein has a reduced rate of recycling or transporter internalization compared to a wild-type form of the membrane transport protein, said process comprising:

- (a) determining the level of the labeled GLUT4 protein or labeled mutant GLUT4 protein at the plasma membrane of a cell expressing the labeled GLUT4 protein or labeled mutant GLUT4 protein using a method comprising:
20
 - (i) contacting the cell with a ligand that binds to the label for a time and under conditions sufficient for the ligand to bind to the labeled GLUT4 protein or labeled mutant GLUT4 protein; and
 - (ii) determining the level of ligand bound to the labeled GLUT4 protein or
25 labeled mutant GLUT4 protein;
- (b) determining the level of membrane transport protein within another cell expressing the labeled GLUT4 protein or labeled mutant GLUT4 protein using a method comprising:
30
 - (i) permeabilizing or disrupting the other cell;
 - (ii) contacting the labeled GLUT4 protein or labeled mutant GLUT4 protein within the cell with a ligand that binds to the label for a time and under conditions sufficient for the ligand to bind to the labeled GLUT4 protein or labeled mutant GLUT4 protein;
 - (iii) determining the level of ligand bound to the labeled GLUT4 protein or
35 labeled mutant GLUT4 protein; and

- (c) comparing the level of ligand detected at (a) (ii) and (b) (iii) to determine the level of the labeled GLUT4 protein or labeled mutant GLUT4 protein at the plasma membrane relative to the total level of labeled GLUT4 protein or labeled mutant GLUT4 protein.

5

The present invention additionally provides a process for determining the level of a labeled GLUT4 protein or a labeled mutant GLUT4 protein translocated to the plasma membrane of a cell that is resistant to insulin induced GLUT4 translocation, wherein said labeled mutant GLUT4 protein has a reduced rate of recycling or transporter internalization compared to a wild-type form of the membrane transport protein, said process comprising:

- (a) contacting a plurality of cells expressing a labeled GLUT4 protein or a labeled mutant GLUT4 protein with an amount of insulin sufficient to induce resistance to insulin induced translocation for a time and under conditions sufficient to induce resistance to insulin induced GLUT4 translocation in the cell, wherein the cells are contacted with insulin in the absence of serum and wherein the cells are contacted with insulin for a period of time from about 24 hours to about 48 hours;
- (b) determining the level of the labeled GLUT4 protein or labeled mutant GLUT4 protein at the plasma membrane of a cell (a) using a method comprising:
- (i) contacting the cell with a ligand that binds to the label for a time and under conditions sufficient for the ligand to bind to the labeled GLUT4 protein or labeled mutant GLUT4 protein; and
- (ii) determining the level of ligand bound to the labeled GLUT4 protein or labeled mutant GLUT4 protein;
- (c) determining the level of labeled GLUT4 protein or labeled mutant GLUT4 protein in another cell (a) using a method comprising:
- (i) permeabilizing or disrupting the other cell;
- (ii) contacting the labeled GLUT4 protein or labeled mutant GLUT4 protein within the cell with a ligand that binds to the label for a time and under conditions sufficient for the ligand to bind to the labeled GLUT4 protein or labeled mutant GLUT4 protein;
- (iii) determining the level of ligand bound to the labeled GLUT4 protein or labeled mutant GLUT4 protein; and
- (d) comparing the level of ligand detected at (b) (ii) and (c) (iii) to determine the level of the labeled GLUT4 protein or labeled mutant GLUT4 protein at the

plasma membrane relative to the total level of labeled GLUT4 protein or labeled mutant GLUT4 protein.

The present invention additionally provides a process for determining the level of recycling of a membrane transport protein in a cell comprising:

- (a) determining the level of the membrane transport protein translocated to the plasma membrane of a cell using the process of the invention;
- (b) determining the level of the membrane transport protein translocated to the plasma membrane of another cell using the process of the invention, wherein the other cell is cultured for a longer period of time than the cell (a); and
- (c) comparing the level of the membrane transport protein translocated to the plasma membrane at (a) and (b) to determine the level of recycling of the membrane transport protein in the cell.

The present invention additionally provides a process for determining a change in the level of recycling of a membrane transport in a cell comprising:

- (a) determining the level of the membrane transport protein translocated to the plasma membrane of a cell using the process of the invention;
- (b) determining the level of the membrane transport protein translocated to the plasma membrane of another cell using the process of the invention, wherein the other cell is cultured for a longer period of time than the cell (a); and
- (c) comparing the level of the membrane transport protein translocated to the plasma membrane at (a) and (b),

wherein a change in the level of the membrane transport protein translocated to the plasma membrane indicates a change in the level of recycling of a membrane transport protein.

The present invention additionally provides a process for determining a mutation in a nucleic acid encoding a mutant membrane transport protein, wherein said mutation modulates translocation of said membrane transport protein, said method comprising:

- (i) determining the level of the mutant membrane transport protein translocated to the plasma membrane of a cell using the process of the invention; and
- (ii) determining the level of the wild-type form of the membrane transport protein translocated to the plasma membrane of a cell using the process of the invention,

wherein an enhanced or suppressed level of translocation of the membrane transport protein at (a) compared to (b) indicates that the nucleic acid comprises a mutation that modulates the level of level of translocation of the membrane transport protein to the plasma membrane.

5

The present invention additionally provides a process for determining an agent that modulates translocation of a membrane transport protein to the plasma membrane of a cell, said process comprising:

- 10 (a) determining the level of a membrane transport protein translocated to the plasma membrane of a cell in the absence of a candidate agent by performing the process of the invention;
- (b) determining the level of the membrane transport protein translocated to the plasma membrane of a cell in the presence of the candidate agent by performing the process of the invention, wherein a difference in the level of the membrane transport protein translocated to the plasma membrane of a cell at (a) compared to (b) indicates that the candidate agent modulates translocation of the membrane transport protein.
- 15 (c) optionally, determining the structure of the candidate agent;
- (d) optionally, providing the name or structure of the candidate agent; and
- 20 (e) optionally, providing, the candidate agent.

The present invention further provides a process for determining a candidate compound for the treatment of insulin resistance comprising:

- 25 (a) determining the level of the labeled GLUT4 protein or the labeled mutant GLUT4 protein translocated to the plasma membrane of a cell in the absence of a candidate agent by performing the process for determining the level of a labeled GLUT4 protein or a labeled mutant GLUT4 protein translocated to the plasma membrane of a cell that is resistant to insulin induced GLUT4 translocation, wherein said labeled mutant GLUT4 protein has a reduced rate of recycling or transporter internalization compared to a wild-type form of the membrane transport protein; and
- 30 (b) determining the level of the labeled GLUT4 protein or a labeled mutant GLUT4 protein translocated to the plasma membrane of another cell in the presence of the candidate agent by performing the process for determining the level of a labeled GLUT4 protein or a labeled mutant GLUT4 protein translocated to the plasma membrane of a cell that is resistant to insulin induced GLUT4
- 35

- translocation, wherein said labeled mutant GLUT4 protein has a reduced rate of recycling or transporter internalization compared to a wild-type form of the membrane transport protein and wherein a candidate agent that enhances the level of translocation of the labeled GLUT4 protein or a labeled mutant GLUT4 protein is a candidate agent for the treatment of insulin resistance.
- 5 (c) optionally, determining the structure of the candidate agent;
(d) optionally, providing the name or structure of the candidate agent; and
(e) optionally, providing, the candidate agent.
- 10 For example, the insulin resistance is associated with diabetes, e.g., the diabetes is type II diabetes.

The present invention additionally provides a process for manufacturing a medicament for the treatment of insulin resistance comprising:

- 15 (a) determining a candidate compound for the treatment of insulin resistance using a process comprising:
- 20 (i) determining the level of the labeled GLUT4 protein or the labeled mutant GLUT4 protein translocated to the plasma membrane of a cell in the absence of a candidate agent by performing the process for determining the level of a labeled GLUT4 protein or a labeled mutant GLUT4 protein translocated to the plasma membrane of a cell that is resistant to insulin induced GLUT4 translocation, wherein said labeled mutant GLUT4 protein has a reduced rate of recycling or transporter internalization compared to a wild-type form of the membrane transport protein; and
- 25 (ii) determining the level of the labeled GLUT4 protein or a labeled mutant GLUT4 protein translocated to the plasma membrane of another cell in the presence of the candidate agent by performing the process for determining the level of a labeled GLUT4 protein or a labeled mutant GLUT4 protein translocated to the plasma membrane of a cell that is resistant to insulin induced GLUT4 translocation, wherein said labeled mutant GLUT4 protein has a reduced rate of recycling or transporter internalization compared to a wild-type form of the membrane transport protein and wherein a candidate agent that enhances the level of translocation of the labeled GLUT4 protein or a labeled mutant GLUT4 protein is a candidate agent for the treatment of insulin resistance.
- 30
- 35 (b) optionally, isolating the candidate agent;

- (c) optionally, providing the name or structure of the candidate agent;
- (d) optionally, providing the candidate agent; and
- (e) using the candidate agent in the manufacture of a medicament for the treatment of insulin resistance.

5

Brief description of the figures

Figure 1A is a schematic representation of a recombinant GLUT4 protein that is labeled with a HA epitope. Note that when expressed in a cell the HA epitope is within the first extracellular domain of the protein. This location of the HA epitope facilitates
10 detection of the GLUT4 protein when translocated to the plasma membrane without disrupting said plasma membrane.

Figure 1B is a schematic representation showing the various forms of GLUT4 used in the analysis of translocation of GLUT4 to the plasma membrane. WT represents the
15 wild-type form of GLUT4 (SEQ ID NO: 1) TAIL represents a mutant form of GLUT4 in which the residues at the C-terminus of GLUT4 have been mutated (SEQ ID NO: 5); L489,490A represents a mutant form of GLUT4 in which a di-leucine motif at the C-terminal end of GLUT4 has been mutated to a di-Alanine motif (SEQ ID NO: 6); and F5A represents a mutant form of GLUT4 in which the phenylalanine at amino acid
20 number 5 of GLUT4 has been mutated to Alanine (SEQ ID NO: 7), wherein each of these proteins have been labeled with a HA epitope tag (SEQ ID NO: 18) in an intracellular domain, for example, the sequence of a WT, GLUT4 labeled with an HA epitope tag is represented by SEQ ID NO: 3.

25 Figure 1C is a schematic representation of one example of the method of detecting the amount of GLUT4 that has translocated to the plasma membrane. The left hand side of the figure shows a cell that is stained to determine the amount of GLUT4 that has translocated to the membrane. Recombinant GLUT4 labeled with a HA epitope is expressed in the cell; the cell is then fixed and the GLUT4 that has translocated to the
30 plasma membrane is detected with an anti-HA antibody; the cell is then permeabilized with saponin and the anti-HA antibody detected with a fluorescent secondary antibody. The right hand side of the figure shows a cell that is used to determine the total amount of GLUT4 in a cell. Recombinant GLUT4 labeled with a HA epitope is expressed in the cell; the cell is then fixed; and permeabilized with saponin. The HA epitope is then
35 detected with an anti-HA antibody, which is now able to enter the cell. The anti-HA epitope is then detected with a fluorescent secondary antibody. Comparing the results

obtained from the two cells shows the amount of GLUT4 that has translocated to the plasma membrane as a function of total GLUT4.

Figure 1D is a copy of a photographic representation showing 3T3-L1 adipocytes
5 expressing HA-GLUT4 WT immunolabeled with an anti-HA or anti-GLUT4 for the detection of HA-GLUT4 or total GLUT4 content respectively.

Figure 1E is a copy of a photographic representation showing an immunoblot on which
cell extracts from 3T3-L1 fibroblasts (F) or 3T3-L1 adipocytes (A) expressing the
10 indicated HA-tagged GLUT4 protein were analyzed using the indicated antibody (left hand side).

Figure 1F is a graphical representation showing the level of expression of each of the
HA-tagged GLUT4 proteins shown in Figure 1C
15

Figure 1G is a copy of a photographic representation of various cells used to analyze
the translocation of GLUT4. The top row of cells are 3T3-L1 fibroblasts and the bottom
row 3T3-L1 adipocytes. From left to right the cells were not transduced (i.e. do not
express a tagged GLUT4); were transduced with a tagged WT, GLUT4; were
20 transduced with a tagged TAIL mutant GLUT4; were transduced with a tagged
L489,490A mutant GLUT4; or were transduced with a tagged F5A mutant GLUT4.

Figure 2A is a graphical representation of the effect of insulin that do not express HA-
tagged GLUT4. The amount of fluorescence detected using the anti-HA antibody (HA)
25 was the same as that detected with a non-relevant (NR) antibody, indicating that the
anti-HA antibody does not non-specifically bind a protein in the cell.

Figure 2B is a graphical representation of the amount of HA tagged GLUT4 detected at
the plasma membrane of 3T3-L1 adipocytes incubated in the presence of 200 nM
30 insulin. Over time, the amount of HA-tagged GLUT4 (squares) detected at the plasma
membrane increased, while the amount of the non-relevant protein (triangles) remained
constant. This indicates that insulin induces GLUT4 translocation to the plasma
membrane.

Figure 2C is a graphical representation of the percentage of total GLUT4 in a cell that
has translocated the plasma membrane in the presence of 200 nM insulin. Using the
35

method described herein the amount of HA tagged GLUT4 that was translocated to the plasma membrane in the presence of insulin was determined relative to the total HA-tagged GLUT4 in a cell.

- 5 Figure 2D is a graphical representation of the percentage of total GLUT4 in a cell that has translocated to the plasma membrane in the presence of various concentrations of insulin. Using the method described herein the effect of insulin concentration on the amount of HA-tagged GLUT4 translocation to the plasma membrane relative to the total HA-tagged GLUT4 was determined (triangle). In the presence of wortmannin
10 (squares) insulin induced translocation of GLUT4 was almost totally abrogated.

- Figure 3A is a graphical representation showing the amount of a HA-tagged form of GLUT4 (from left to right: WT; TAIL; L489; 490A; and F5A) detected at the plasma membrane of 3T3-L1 fibroblasts at relative to the total HA-tagged form of GLUT4.
15 Clearly GLUT4 translocation is induced by insulin in fibroblasts.

- Figure 3B is a graphical representation showing the percentage of a HA-tagged form of GLUT4 (from left to right: WT; TAIL; L489; 490A; and F5A) at the plasma membrane of 3T3-L1 adipocytes in the presence of 200 nM insulin. Interestingly, the L489;
20 L490A and F5A mutants, which are believed to be impaired in their internalization/recycling, show an increase in adipocytes compared with fibroblasts (Figure 3A).

- Figure 4 is a graphical representation showing the internalization kinetics of HA-
25 GLUT4 in 3T3-L1 adipocytes. Adipocytes expressing the indicated GLUT4 molecule were incubated for 20 min with 200 nM insulin at 37°C and for 1 h with anti-HA antibody on ice. Excess antibody was washed away, and cells were incubated for the indicated periods at 37°C in the presence of either 100 nM wortmannin, to measure GLUT4 internalization in the basal state, or 200 nM insulin. Cells were exposed to
30 fixative and incubated with fluorescent secondary antibody in the absence of permeabilizing agent to allow measurement of the time-dependent disappearance of anti-HA-labeled GLUT4 from the cell surface.

- Figure 5A is a copy of a photographic representation showing the subcellular
35 localization of HA-tagged GLUT4 in adipocytes incubated for 2 hours with 200 nM

insulin and subsequently for 2 hours without insulin and then 20 minutes without insulin.

Figure 5B is a copy of a photographic representation showing the subcellular
5 localization of HA-tagged GLUT4 in adipocytes incubated for 2 hours with 200 nM insulin and subsequently for 2 hours with insulin and then 20 minutes without insulin.

Figure 5C is a copy of a photographic representation showing the subcellular
localization of HA-tagged GLUT4 in adipocytes incubated for 2 hours with 200 nM
10 insulin and anti-HA antibody and subsequently for 2 hours without insulin and anti-HA antibody and then 20 minutes without insulin.

Figure 5D is a copy of a photographic representation showing the subcellular
localization of HA-tagged GLUT4 in adipocytes incubated for 2 hours with 200 nM
15 insulin and anti-HA antibody and subsequently for 2 hours without insulin and anti-HA antibody and then 20 minutes with insulin.

Figure 5E shows graphical representations showing levels of antibody uptake in
fibroblasts or adipocytes as indicated at the left hand-side of the figure expressing the
20 indicated HA-GLUT4 protein. Cells were incubated with (squares) or without (triangles) 200nM insulin for 20 min, after which anti-HA antibody was added. Cells were incubated for up to 180 minutes, fixed permeabilized and incubated with a fluorescently labeled secondary antibody. The level of anti-HA antibody taken up by the cells is expressed as a percentage of total post-fixation anti-HA labeling.

25
Figure 6A is a graphical representation demonstrating the existence of a non-recycling pool of HA-GLUT4 WT in a cell. Cells were incubated in the presence of insulin for an extended period of time (180min) and the level of HA-GLUT4 at the plasma membrane relative to the total level detected in the cell was determined.

30
Figure 6B is a graphical representation showing the level of HA-GLUT4 in the cells used to determine the level of HA-GLUT4 in the cell (Figure 6A) following an additional incubation with fixative.

35
Figure 6C is a graphical representation showing the level of HA-GLUT4 detected at the plasma membrane of cells in which the level of HA-GLUT4 at the plasma membrane

was previously determined (Figure 6A) following an additional incubation with an anti-HA antibody (and detection of the level of bound anti-HA antibody).

5 Figure 6D is a graphical representation showing the level of HA-GLUT4 detected within cells previously fixed and permeabilized following an additional incubation with an anti-HA antibody (and detection of the level of bound anti-HA antibody).

10 Figure 6E is a graphical representation showing the relative level (percentage of total) level of HA-GLUT4 WT detected at the plasma membrane of a cell using various concentrations of anti-HA antibody.

15 Figure 6F is a graphical representation showing the relative level (percentage of total) of HA-GLUT4 WT detected at the plasma membrane of a cell following a 2 hour incubation in the presence of cycloheximide.

20 Figure 6G is a graphical representation showing the effect of endosomal pH on the binding of the anti-HA antibody to HA-GLUT4. Cells were incubated for 30 min at 37°C in hypertonic medium (0.45 M sucrose, pH 7.4), on ice with antibody in the same medium, and at 37°C in hypertonic buffer at pH 7.4 or pH 5.5 in the absence of antibody. Release of antibody from the PM at neutral or endosomal pH was determined by incubating fixed non-permeabilized cells with fluorescent secondary antibody.

25 Figure 6H is a graphical representation showing the effect of incubating a cell in the presence of insulin for an extended period of time. Cells were incubated in the presence of 200nM insulin for up to 3 hours and the relative level (percentage of total) of HA-GLUT4 at the plasma membrane determined.

30 Figure 7 shows graphical and photographic representations showing GLUT4 recycling during the differentiation of 3T3-L1 fibroblasts into adipocytes. FIG. 5. Cells were analyzed at different stages during differentiation as indicated. After incubation for 18 h in medium containing fetal bovine serum and for 2 h in the absence of serum, the cells were incubated in the continuous presence of anti-HA antibody as described for Fig. 4. Parallel cultures were incubated similarly but analyzed by immunofluorescence confocal microscopy (left microscopy panels). Non-infected cells were analyzed for endogenous
35 GLUT4 and lipid droplet content during differentiation (right microscopy panels). Bottom

right microscopy panels show Z section image of the cells. White dotted lines mark the contours of the cells.

- Figure 8A is a graphical representation showing a correlation between insulin concentration and the size of the non-recycling GLUT4 pool in 3T3-L1 adipocytes. 3T3-L1 adipocytes expressing HA-GLUT WT or HA-GLUT TRAIL were incubated at 37°C with anti-HA antibody and the indicated concentration of insulin and the level of cell associated HA antibody was determined.
- 10 Figure 8B is a graphical representation showing 3T3-L1 adipocytes expressing HA-GLUT4 WT or HA-GLUT4 TAIL that were incubated for 20 min at 37°C with 0.032, 0.24, 3.2, 15 or 200 nM insulin and amounts of GLUT4 at the PM were determined and expressed as percentage of maximal insulin-induced GLUT4 translocation.
- 15 Figure 8C is a copy of a photographic representation showing HA-GLUT4-expressing 3T3-L1 adipocytes incubated for 3 h with anti-HA antibody and the indicated concentrations of insulin. Cells were fixed, permeabilized, incubated with fluorescent secondary antibody and analyzed by confocal immunofluorescence microscopy.
- 20 Figure 9 is a graphical representation showing the translocation of HA-GLUT4 in 3T3-L1 adipocytes grown and differentiated in a 384-well plate compared to cells grown and differentiated in a Petri dish and transferred to a 384-well plate. Axes are time of insulin exposure (min, X-axis) and percentage of total HA-GLUT4 detected at the plasma membrane (Y-axis).
- 25 Figure 10 is a graphical representation showing the effect of amino acid concentration on the level of HA-GLUT4 translocated to the plasma membrane of a cell. HA-GLUT4 expressing adipocytes were serum starved for 2 hours in Krebs Ringer Phosphate buffer or in the same buffer supplemented with amino acid concentrations used in Dulbecco's modified eagle medium of Gibco (2x amino acids) or with half of the amino acid concentration (1x amino acids) as indicated. Axes are time of insulin exposure (min, X-axis) and percentage of total HA-GLUT4 detected at the plasma membrane (Y-axis).
- 30
- 35 Figure 11 is a graphical representation showing the effect of insulin and sucrose on HA-GLUT4 translocation. 3T3-L1 adipocytes expressing HA-GLUT4 WT were serum

starved for 2 hours at 37°C. Following 20 minutes of acute insulin stimulation with 200nM, cells were incubated for additional 2 hours in serum free medium supplemented with 0.2% BSA and 0.3 or 0.6M sucrose as indicated. After post-fixation anti-HA immunolabeling the amount of cell surface HA-GLUT4 levels was
5 determined. Axes are insulin concentration (nM, X-axis) and percentage of total HA-GLUT4 detected at the plasma membrane (Y-axis).

Figure 12A is a graphical representation showing the induction of insulin resistance in 3T3-L1 adipocytes. 3T3-L1 adipocytes retrovirally infected with HA-GLUT4 were
10 incubated 24 hours or 48 hours either with 600nM insulin or with medium alone. After this chronic insulin stimulation for the indicated periods of time, cells were washed and 200 nM insulin added for additional 10 or 30 minutes and cell surface levels of HA-GLUT4 were measured using the fluorescence based assay. Treatment groups are indicated. Y axis shows the percentage of total HA-GLUT4 detected at the plasma
15 membrane.

Figure 12B is a graphical representation showing the induction of insulin resistance in 3T3-L1 adipocytes expressing a mutant GLUT4. 3T3-L1 adipocytes retrovirally infected with HA-GLUT4 TAIL mutant were incubated 24 hours or 48 hours either
20 with 600nM insulin or with medium alone. After this chronic insulin stimulation for the indicated periods of time, cells were washed and 200 nM insulin added for additional 10 or 30 minutes and cell surface levels of HA-GLUT4 TAIL were measured using the fluorescence based assay. Treatment groups are indicated. Y axis shows the percentage of total HA-GLUT4 detected at the plasma membrane.

25
Figure 13 is a graphical representation showing the effect of wortmannin on acute and chronic insulin induced GLUT4 translocation. HA-GLUT4 expressing 3T3-L1 adipocytes were grown in 96 well plates, incubated for 2 hours or overnight in medium supplemented with 10% fetal calf serum or no serum. 200nM insulin in case of acute
30 stimulation and 600nM insulin in case of chronic stimulation were used (as indicated). Following overnight stimulation cells were washed and 200nM fresh insulin was added for 10 or 30 min. Both medium conditions were tested in the presence and absence of 100nM wortmannin. Y axis shows the percentage of total HA-GLUT4 detected at the plasma membrane.

35

Detailed description of the preferred embodiments

The present invention provides a process for determining the level of a membrane transport protein translocated to the plasma membrane of a cell, said method comprising:

- 5 (a) determining the level of a membrane transport protein at the plasma membrane using a method comprising:
 - (i) contacting the membrane transport protein with a ligand that binds to an extracellular domain of the membrane transport protein for a time and under conditions sufficient for the ligand to bind to the membrane transport protein; and
 - 10 (ii) determining the level of ligand bound to the membrane transport protein;
- (b)
 - (i) permeabilizing or disrupting the plasma membrane of a cell and contacting the membrane transport protein within the cell with a ligand that binds to an extracellular domain of the membrane transport protein for a time and under conditions sufficient for the ligand to bind to the
 - 15 membrane transport protein; and
 - (ii) determining the level of ligand bound to the membrane transport protein within the cell; and
- (c) comparing the level of ligand detected at (a) (ii) and (b) (ii) to determine the level of the membrane transport protein at the plasma membrane relative to the
- 20 level of the membrane transport protein inside the cell.

For example, a ligand of a membrane transport protein that binds to an extracellular domain of the membrane transport protein is, for example, an antibody. Antibodies that bind an extracellular domain of a membrane protein are known in the art. For

25 example, monoclonal antibody mAb5 or mAb263 that specifically bind an extracellular region of the growth hormone receptor protein (available from AGEN Limited, Acacia Ridge, Queensland, Australia). A polyclonal antibody that bind to an extracellular domain of GLUT2 is available from Alpha Diagnostics International Inc., San Antonio, TX, USA. An antibody that binds to an extracellular domain of GLUT1 is described in

30 Carbó *et al.*, *Clinical and Experimental Pharmacology and Physiology* 30: 64, 2003. Alternatively, the antibody or ligand is produced by a method known in the art and/or described herein.

Membrane transport proteins

35 As used herein, the term "membrane transport protein" shall be taken to mean a peptide, polypeptide or protein that catalyzes the movement of a molecule across a

membrane, whether this movement is by diffusion (simple or facilitated) or active transport. Membrane transport proteins in the present context exist as intracellular proteins and are capable of being membrane-localized. Such a protein may be, for example, a channel, a transporter, an ATP pump, a symporter or an antiporter. The term "membrane transport protein" shall be taken to include mutant forms of a membrane transport protein (for example, a mutant form of a membrane transport protein capable of translocating to the plasma membrane of a cell) and/or a labeled membrane transport protein. For example, a labeled membrane transport protein described herein.

10

For example, a membrane transport protein useful in performance of the invention is a protein from a family of proteins selected from the group consisting of amino acid/auxin permease (AAP) family, amino acid-polyamine-organocation (APC) family, cation-chloride cotransporter (CCC) family, hydroxy/aromatic amino acid permease (HAAAP) family, bile acid:Na⁺ symporter (BASS) family, arsenical resistance-3 (ARC3) family, monovalent cation:proton antiporter-1 (CPA1) family, monovalent cation:proton antiporter-2 (CPA2) family, Na⁺-transporting carboxylic acid decarboxylase (NaT-DC) family, citrate-Mg²⁺:H⁺ (MitM) citrate-Ca²⁺:H⁺ (CitH) symporter (CitMHS) family, C₄-dicarboxylate uptake (Dcu) family, lactate permease (LctP) family, NhaB Na⁺:H⁺ antiporter (NhaB) family, NhaC Na⁺:H⁺ antiporter (NhaC) family, arsenite-antimonite (ArsB) efflux family, divalent anion:Na⁺ symporter (DASS) family, tripartite ATP-independent periplasmic transporter (TRAP-T) family, C₄-dicarboxylate uptake C (DcuC) family, NhaD Na⁺:H⁺ antiporter (NhaD) family, p-aminobenzyol-glutamate transporter (AbgT) family, gluconate:H⁺ symporter (GntP) family, L-lysine exporter (LysE) family, major facilitator superfamily (MFS), proton-dependent oligopeptide transporter (POT) family, organo-anion transporter (OAT) family, folate-biopterin transporter (FBT) family, PTS galactitol (Gat) family, PTS L-ascorbate (L-Asc) family, PTS glucose-glucoside (Glc) family, PTS fructose-mannitol (Fru) family, voltage-gated ion channel (VIC) family, glutamate gated ion channel (GIC) family of neurotransmitter receptors, animal inward rectifier K⁺ channel (RIR-CaC) family, ryanodine-inositol 1, 4, 5-triphosphate receptor Ca²⁺ channel (RIR-CaC) family and K⁺ transporter (Trk) family. Information concerning the structure and/or function of a membrane transport protein (e.g., a membrane transport protein from a family described *supra*) is found in, for example, the Transport Classification Database available from University of California, San Diego, La Jolla, Ca, USA.

For example, the membrane transport protein is a human membrane transport protein. For example, a human membrane transport protein selected from the group consisting of a human annexin, a human ATP-binding cassette transporter, a human ATPase, a human calcium channel, a human potassium channel, a human sodium channel and a
5 human solute carrier.

For example, the membrane transport protein is a protein that translocates to a plasma membrane of a cell under normal physiological conditions, or following stimulation by a condition or agent, such as, for example, glucose or insulin. Preferably the membrane
10 transport protein is, for example, an ABC transporter protein, a P class ATP pump, a F class ATP pump, a V class ATP pump, a Cl^- channel, a H^+ channel and Ca^{++} channel, a K^+ channel, an uniporter a symporter or an antiporter. For example, the membrane transport protein is a membrane transport protein selected from the group consisting of ABC1, ABCA2, ABCA3, ABCR, ABCA5, ABCA6, ABCA7, ABCA8, ABCA9,
15 ABCA10, ABCA12, ABCA13, PGY1, TAP1, TAP2, PGY3, ABCB5, ABCB6, ABC7, M-ABC1, ABCB9, ABCB10, BSEP, MRP1, MRP2, MRP3, MRP4, MRP5, MRP6, CFTR, SUR1, SUR2, ABCC10, ABCC11, ABCC12, ABCC13, ALD, ALDL1, ABCD2, PXMP1, PXMP1L, RNASEL1, ABC50, ABCF2, ABCF3, ABCG1, ABCG2, ABCG4, ABCG5, ABCG8, KCNA1, CACNL1A4, KCNQ2, KCNQ3, SCN1B,
20 CHRNA4, GLRA1, KCNE1, KCNQ4, SCN4A, CACNL1A3, CLCN1, CNCN1, RYR1, CHRNA1, KCNQ1, HERG, SCN5A, KCNE1, SCN5A, KCNE1, GLUT1, GLUT2, GLUT3, GLUT4, GLUT5, GLUT6, GLUT7, GLUT8, GLUT9, GLUT10, GLUT11, GLUT12, HMIT and GLUT14.

25 As used herein, the nomenclature for GLUT proteins and HMIT is described by Joost *et al*, 2001, *Am. J. Physiol. Endocrinol. Metab.* 282: E974-E976, 2002.

In an example of the invention, the membrane transport protein is a glucose transport protein or a facilitated glucose transport protein (GLUT). As used herein the term
30 "glucose transport protein" or "facilitated glucose transport protein" or "GLUT" shall be taken to mean a member of the SCLC2A family of solute carrier proteins. Individual member of this family have similar predicted secondary structures with 12 transmembrane domains. Both N and C-termini are predicted to be cytoplasmic. There is a large extracellular domain between transmembrane region 1 and transmembrane
35 region 2 and a large cytoplasmic domain between transmembrane region 6 and transmembrane region 7.

GLUT isoforms differ in their tissue expression, substrate specificity and kinetic characteristics. Table 1 outlines many of the characteristics of GLUT isoforms.

Table 1: GLUT isoforms

GLUT Isoform	Characteristics
GLUT1	mediates glucose transport into red cells, and throughout the blood brain barrier. It is ubiquitously expressed and transports glucose in most cells
GLUT2	provides glucose to the liver and pancreatic cells
GLUT3	the main glucose transporter in neurons
GLUT4	primarily expressed in muscle and adipose tissue and regulated by insulin
GLUT5	transports fructose in intestine and testis
GLUT6	highly expressed in brain, spleen, and leukocytes.
GLUT8	High levels are found in adult testis and placenta
GLUT9	expressed in kidney, liver, placenta, lung, blood leukocytes, heart, and skeletal muscle
GLUT10	widely expressed with highest levels in liver and pancreas
GLUT11	expressed in heart and skeletal muscle
GLUT12	expressed in skeletal muscle, adipose tissue, and small intestine
GLUT13	(aka. H ⁺ myo-inositol transporter, HMIT) predominantly expressed in brain

For example, the process of the invention is performed with a GLUT protein selected from the group consisting of a GLUT1 protein, a GLUT2 protein, a GLUT3 protein, a GLUT4 protein, a GLUT5 protein, a GLUT6 protein, a GLUT7 protein, a GLUT8 protein, a GLUT9 protein, a GLUT10 protein, a GLUT11 protein, a GLUT12 protein, a GLUT13 (HMIT) protein, a GLUT14 protein.

As used herein, the term "GLUT1 protein" shall be taken to mean a protein that comprises an amino acid sequence at least about 80% identical to the sequence set forth in SEQ ID NO: 12. For example, the protein comprises an amino acid sequence at least about 85% or at least about 90% or at least about 95% or at least about 98% or at least about 99% identical to the amino acid sequence set forth in SEQ ID NO: 12.

15

In one example, the GLUT1 protein is a human GLUT1 protein.

Alternatively, or in addition, a GLUT 1 protein is a protein encoded by a nucleic acid that comprises a nucleotide sequence at least about 80% identical to the nucleotide sequence set forth in SEQ ID NO: 11. For example, the nucleic acid comprises a
5 nucleotide sequence at least about 85% or at least about 90% or at least about 95% or at least about 98% or at least about 99% identical to the nucleotide sequence set forth in SEQ ID NO: 11.

As used herein, the term "GLUT2 protein" shall be taken to mean a protein that
10 comprises an amino acid sequence at least about 80% identical to the sequence set forth in SEQ ID NO: 38. For example, the protein comprises an amino acid sequence at least about 85% or at least about 90% or at least about 95% or at least about 98% or at least about 99% identical to the amino acid sequence set forth in SEQ ID NO: 38.

15 In one example, the GLUT2 protein is a human GLUT2 protein.

Alternatively, or in addition, a GLUT2 protein is a protein encoded by a nucleic acid that comprises a nucleotide sequence at least about 80% identical to the nucleotide sequence set forth in SEQ ID NO: 37. For example, the nucleic acid comprises a
20 nucleotide sequence at least about 85% or at least about 90% or at least about 95% or at least about 98% or at least about 99% identical to the nucleotide sequence set forth in SEQ ID NO: 37.

As used herein, the term "GLUT3 protein" shall be taken to mean a protein that
25 comprises an amino acid sequence at least about 80% identical to the sequence set forth in SEQ ID NO: 40. For example, the protein comprises an amino acid sequence at least about 85% or at least about 90% or at least about 95% or at least about 98% or at least about 99% identical to the amino acid sequence set forth in SEQ ID NO: 40.

30 In one example, the GLUT3 protein is a human GLUT3 protein.

Alternatively, or in addition, a GLUT3 protein is a protein encoded by a nucleic acid that comprises a nucleotide sequence at least about 80% identical to the nucleotide sequence set forth in SEQ ID NO: 39. For example, the nucleic acid comprises a
35 nucleotide sequence at least about 85% or at least about 90% or at least about 95% or at

least about 98% or at least about 99% identical to the nucleotide sequence set forth in SEQ ID NO: 39.

As used herein, the term "GLUT4 protein" shall be taken to mean a protein that
5 comprises an amino acid sequence at least about 80% identical to the sequence set forth in SEQ ID NO: 2. For example, the protein comprises an amino acid sequence at least about 85% or at least about 90% or at least about 95% or at least about 98% or at least about 99% identical to the amino acid sequence set forth in SEQ ID NO: 2.

10 In one example, the GLUT4 protein is a human GLUT4 protein.

Alternatively, or in addition, a GLUT 4 protein is a protein encoded by a nucleic acid that comprises a nucleotide sequence at least about 80% identical to the nucleotide sequence set forth in SEQ ID NO: 1. For example, the nucleic acid comprises a
15 nucleotide sequence at least about 85% or at least about 90% or at least about 95% or at least about 98% or at least about 99% identical to the nucleotide sequence set forth in SEQ ID NO: 1.

As used herein, the term "GLUT5 protein" shall be taken to mean a protein that
20 comprises an amino acid sequence at least about 80% identical to the sequence set forth in SEQ ID NO: 2. For example, the protein comprises an amino acid sequence at least about 85% or at least about 90% or at least about 95% or at least about 98% or at least about 99% identical to the amino acid sequence set forth in SEQ ID NO: 42.

25 In one example, the GLUT5 protein is a human GLUT5 protein.

Alternatively, or in addition, a GLUT5 protein is a protein encoded by a nucleic acid that comprises a nucleotide sequence at least about 80% identical to the nucleotide sequence set forth in SEQ ID NO: 41. For example, the nucleic acid comprises a
30 nucleotide sequence at least about 85% or at least about 90% or at least about 95% or at least about 98% or at least about 99% identical to the nucleotide sequence set forth in SEQ ID NO: 41.

As used herein, the term "GLUT6 protein" shall be taken to mean a protein that
35 comprises an amino acid sequence at least about 80% identical to the sequence set forth in SEQ ID NO: 44. For example, the protein comprises an amino acid sequence at least

about 85% or at least about 90% or at least about 95% or at least about 98% or at least about 99% identical to the amino acid sequence set forth in SEQ ID NO: 44.

In one example, the GLUT6 protein is a human GLUT6 protein.

5

Alternatively, or in addition, a GLUT6 protein is a protein encoded by a nucleic acid that comprises a nucleotide sequence at least about 80% identical to the nucleotide sequence set forth in SEQ ID NO: 43. For example, the nucleic acid comprises a nucleotide sequence at least about 85% or at least about 90% or at least about 95% or at least about 98% or at least about 99% identical to the nucleotide sequence set forth in SEQ ID NO: 43.

As used herein, the term "GLUT7 protein" shall be taken to mean a protein that comprises an amino acid sequence at least about 80% identical to the sequence set forth in SEQ ID NO: 46. For example, the protein comprises an amino acid sequence at least about 85% or at least about 90% or at least about 95% or at least about 98% or at least about 99% identical to the amino acid sequence set forth in SEQ ID NO: 46.

15

In one example, the GLUT7 protein is a human GLUT7 protein.

20

Alternatively, or in addition, a GLUT7 protein is a protein encoded by a nucleic acid that comprises a nucleotide sequence at least about 80% identical to the nucleotide sequence set forth in SEQ ID NO: 45. For example, the nucleic acid comprises a nucleotide sequence at least about 85% or at least about 90% or at least about 95% or at least about 98% or at least about 99% identical to the nucleotide sequence set forth in SEQ ID NO: 45.

25

As used herein, the term "GLUT8 protein" shall be taken to mean a protein that comprises an amino acid sequence at least about 80% identical to the sequence set forth in SEQ ID NO: 48. For example, the protein comprises an amino acid sequence at least about 85% or at least about 90% or at least about 95% or at least about 98% or at least about 99% identical to the amino acid sequence set forth in SEQ ID NO: 48.

30

In one example, the GLUT8 protein is a human GLUT8 protein.

35

Alternatively, or in addition, a GLUT8 protein is a protein encoded by a nucleic acid that comprises a nucleotide sequence at least about 80% identical to the nucleotide sequence set forth in SEQ ID NO: 47. For example, the nucleic acid comprises a nucleotide sequence at least about 85% or at least about 90% or at least about 95% or at least about 98% or at least about 99% identical to the nucleotide sequence set forth in SEQ ID NO: 4.

As used herein, the term "GLUT9 protein" shall be taken to mean a protein that comprises an amino acid sequence at least about 80% identical to the sequence set forth in SEQ ID NO: 50. For example, the protein comprises an amino acid sequence at least about 85% or at least about 90% or at least about 95% or at least about 98% or at least about 99% identical to the amino acid sequence set forth in SEQ ID NO: 50.

In one example, the GLUT9 protein is a human GLUT9 protein.

Alternatively, or in addition, a GLUT9 protein is a protein encoded by a nucleic acid that comprises a nucleotide sequence at least about 80% identical to the nucleotide sequence set forth in SEQ ID NO: 49. For example, the nucleic acid comprises a nucleotide sequence at least about 85% or at least about 90% or at least about 95% or at least about 98% or at least about 99% identical to the nucleotide sequence set forth in SEQ ID NO: 49.

As used herein, the term "GLUT10 protein" shall be taken to mean a protein that comprises an amino acid sequence at least about 80% identical to the sequence set forth in SEQ ID NO: 52. For example, the protein comprises an amino acid sequence at least about 85% or at least about 90% or at least about 95% or at least about 98% or at least about 99% identical to the amino acid sequence set forth in SEQ ID NO: 52.

In one example, the GLUT10 protein is a human GLUT10 protein.

Alternatively, or in addition, a GLUT10 protein is a protein encoded by a nucleic acid that comprises a nucleotide sequence at least about 80% identical to the nucleotide sequence set forth in SEQ ID NO: 51. For example, the nucleic acid comprises a nucleotide sequence at least about 85% or at least about 90% or at least about 95% or at least about 98% or at least about 99% identical to the nucleotide sequence set forth in SEQ ID NO: 51.

As used herein, the term "GLUT11 protein" shall be taken to mean a protein that comprises an amino acid sequence at least about 80% identical to the sequence set forth in SEQ ID NO: 54. For example, the protein comprises an amino acid sequence at least
5 about 85% or at least about 90% or at least about 95% or at least about 98% or at least about 99% identical to the amino acid sequence set forth in SEQ ID NO: 54.

In one example, the GLUT11 protein is a human GLUT11 protein.

10 Alternatively, or in addition, a GLUT11 protein is a protein encoded by a nucleic acid that comprises a nucleotide sequence at least about 80% identical to the nucleotide sequence set forth in SEQ ID NO: 53. For example, the nucleic acid comprises a nucleotide sequence at least about 85% or at least about 90% or at least about 95% or at least about 98% or at least about 99% identical to the nucleotide sequence set forth in
15 SEQ ID NO: 53.

As used herein, the term "GLUT12 protein" shall be taken to mean a protein that comprises an amino acid sequence at least about 80% identical to the sequence set forth in SEQ ID NO: 56. For example, the protein comprises an amino acid sequence at least
20 about 85% or at least about 90% or at least about 95% or at least about 98% or at least about 99% identical to the amino acid sequence set forth in SEQ ID NO: 56.

In one example, the GLUT12 protein is a human GLUT12 protein.

25 Alternatively, or in addition, a GLUT12 protein is a protein encoded by a nucleic acid that comprises a nucleotide sequence at least about 80% identical to the nucleotide sequence set forth in SEQ ID NO: 55. For example, the nucleic acid comprises a nucleotide sequence at least about 85% or at least about 90% or at least about 95% or at least about 98% or at least about 99% identical to the nucleotide sequence set forth in
30 SEQ ID NO: 55.

As used herein, the term "GLUT13 protein" or "HMIT" shall be taken to mean a protein that comprises an amino acid sequence at least about 80% identical to the sequence set forth in SEQ ID NO: 57. For example, the protein comprises an amino
35 acid sequence at least about 85% or at least about 90% or at least about 95% or at least

about 98% or at least about 99% identical to the amino acid sequence set forth in SEQ ID NO: 57.

In one example, the GLUT13 or HMIT protein is a human GLUT13 or HMIT protein.

5

Alternatively, or in addition, a GLUT13 or HMIT protein is a protein encoded by a nucleic acid that comprises a nucleotide sequence at least about 80% identical to the nucleotide sequence set forth in SEQ ID NO: 56. For example, the nucleic acid comprises a nucleotide sequence at least about 85% or at least about 90% or at least about 95% or at least about 98% or at least about 99% identical to the nucleotide sequence set forth in SEQ ID NO: 56.

As used herein, the term "GLUT14 protein" shall be taken to mean a protein that comprises an amino acid sequence at least about 80% identical to the sequence set forth in SEQ ID NO: 59. For example, the protein comprises an amino acid sequence at least about 85% or at least about 90% or at least about 95% or at least about 98% or at least about 99% identical to the amino acid sequence set forth in SEQ ID NO: 59.

15

In one example, the GLUT14 protein is a human GLUT14 protein.

20

Alternatively, or in addition, a GLUT14 protein is a protein encoded by a nucleic acid that comprises a nucleotide sequence at least about 80% identical to the nucleotide sequence set forth in SEQ ID NO: 58. For example, the nucleic acid comprises a nucleotide sequence at least about 85% or at least about 90% or at least about 95% or at least about 98% or at least about 99% identical to the nucleotide sequence set forth in SEQ ID NO: 58.

25

In an exemplified form of the invention, the membrane transport protein is a GLUT4 transport protein or a GLUT1 transport protein.

30

In determining whether or not two amino acid sequences fall within the defined percentage identity limits *supra*, those skilled in the art will be aware that it is possible to conduct a side-by-side comparison of the amino acid sequences. In such comparisons or alignments, differences will arise in the positioning of non-identical residues depending upon the algorithm used to perform the alignment. In the present context, references to percentage identities and similarities between two or more amino

35

acid sequences shall be taken to refer to the number of identical and similar residues respectively, between said sequences as determined using any standard algorithm known to those skilled in the art. In particular, amino acid identities and similarities are calculated using software of the Computer Genetics Group, Inc., University Research
5 Park, Maddison, Wisconsin, United States of America, e.g., using the GAP program of Devereaux *et al.*, *Nucl. Acids Res.* 12, 387-395, 1984, which utilizes the algorithm of Needleman and Wunsch, *J. Mol. Biol.* 48, 443-453, 1970. Alternatively, the CLUSTAL W algorithm of Thompson *et al.*, *Nucl. Acids Res.* 22, 4673-4680, 1994, is used to obtain an alignment of multiple sequences, wherein it is necessary or desirable
10 to maximize the number of identical/similar residues and to minimize the number and/or length of sequence gaps in the alignment.

Alternatively, a suite of commonly used and freely available sequence comparison algorithms is provided by the National Center for Biotechnology Information (NCBI)
15 Basic Local Alignment Search Tool (BLAST) (Altschul *et al.* *J. Mol. Biol.* 215: 403-410, 1990), which is available from several sources, including the NCBI, Bethesda, Md.. The BLAST software suite includes various sequence analysis programs including "blastn," that is used to align a known nucleotide sequence with other polynucleotide sequences from a variety of databases and "blastp" used to align a known amino acid
20 sequence with one or more sequences from one or more databases. Also available is a tool called "BLAST 2 Sequences" that is used for direct pairwise comparison of two nucleotide sequences.

As used herein the term "NCBI" shall be taken to mean the database of the National
25 Center for Biotechnology Information at the National Library of Medicine at the National Institutes of Health of the Government of the United States of America, Bethesda, MD, 20894.

In determining whether or not two nucleotide sequences fall within a particular
30 percentage identity limitation recited herein, those skilled in the art will be aware that it is necessary to conduct a side-by-side comparison or multiple alignment of sequences. In such comparisons or alignments, differences may arise in the positioning of non-identical residues, depending upon the algorithm used to perform the alignment. In the present context, reference to a percentage identity between two or more nucleotide
35 sequences shall be taken to refer to the number of identical residues between said sequences as determined using any standard algorithm known to those skilled in the art.

For example, nucleotide sequences may be aligned and their identity calculated using the BESTFIT program or other appropriate program of the Computer Genetics Group, Inc., University Research Park, Madison, Wisconsin, United States of America (Devereaux et al, Nucl. Acids Res. 12, 387-395, 1984). As discussed *supra* BLAST is
5 also useful for aligning nucleotide sequences and determining percentage identity.

In another example of the invention, the membrane transport protein is a cystic fibrosis transmembrane regulator (CFTR) protein. As used herein the term "cystic fibrosis transmembrane regulator protein" or "CFTR" shall be taken to mean a protein that
10 comprises an amino acid sequence at least about 80% identical to the sequence set forth in SEQ ID NO: 36. For example, the protein comprises an amino acid sequence at least about 85% or at least about 90% or at least about 95% or at least about 98% or at least about 99% identical to the amino acid sequence set forth in SEQ ID NO: 36.

15 In one example, the CFTR protein is a human CFTR protein.

Alternatively, or in addition, a CFTR protein is a protein encoded by a nucleic acid that comprises a nucleotide sequence at least about 80% identical to the nucleotide sequence set forth in SEQ ID NO: 35. For example, the nucleic acid comprises a
20 nucleotide sequence at least about 85% or at least about 90% or at least about 95% or at least about 98% or at least about 99% identical to the nucleotide sequence set forth in SEQ ID NO: 35.

In one form of the invention, the CFTR protein is a mutant CFTR protein. For
25 example, a CFTR mutation selected from the group consisting of 1717-1G→A, G542X, W1282X, N1303K, ΔF508, 3849+10kb C→T, 621+1 G→T, R553X, G551D, R117H, R1162X and R334W. For example, a CFTR protein comprising a ΔF508 mutation comprises an amino acid sequence set forth in SEQ ID NO: 61.

30 In another example of the invention the membrane transport protein is a mutant membrane transport protein. As used herein, the term "mutant membrane transport protein" shall be taken to mean a membrane transport protein that comprises one or more amino acid substitutions, insertions or deletions compared to a wild-type form of a membrane transport protein, e.g. a form of a membrane transport protein described
35 *supra*. While it is not a requirement that the mutant membrane transport is functional,

it is beneficial that the membrane transport protein is capable of translocating to a plasma membrane to some degree.

For example, a mutant membrane transport protein has a reduced rate of transporter
5 internalization. As used herein, the term "reduced rate of transporter internalization" shall be taken to mean that has been mutated in such a way that following translocation to the membrane it is not internalized or endocytosed, i.e. translocated away from the membrane at the same rate as the wild-type form of the membrane transport protein, rather it is internalized at a slower rate. For example, a mutant form of GLUT4 that has
10 a reduced rate of transporter internalization includes the L489, 490A mutant (SEQ ID NO: 7) or the F5A mutant (SEQ ID NO: 9). Such a mutant is of use in the process of the present invention as it accumulates at the plasma membrane, effectively amplifying or increasing the level of membrane transport protein detected. Accordingly, such a mutant is useful for detection of a minor change (i.e. increase or decrease) of the
15 translocation of a membrane transport protein, for example, when screening for a modulator of translocation of a membrane transport protein.

In the case of GLUT4, wild-type GLUT4 is more effectively translocated and recycled in the presence of insulin, as would be expected. Accordingly, wild-type GLUT4 is
20 more effective in an assay for determining changes in translocation in the presence and/or absence of insulin, for example, when screening for a compound/agent that modulates GLUT4 translocation in the presence of insulin.

In one example of the invention, the membrane transport protein is a membrane
25 transport protein that is rapidly translocated and recycled, whether that membrane transport protein is a wild-type or mutant form.

Detectable labels

In an example of the invention, the membrane transport protein is labeled. For
30 example, with a detectable label. Accordingly, the present invention provides a process for determining the level of a labeled membrane transport protein translocated to the plasma membrane of a cell expressing the labeled membrane transport protein, said process comprising:

- (a) determining the level of the labeled membrane transport protein at the plasma
35 membrane of a cell using a method comprising:

- (i) contacting the cell with a ligand that binds to the label for a time and under conditions sufficient for the ligand to bind the label; and
 - (ii) determining the level of ligand bound to the labeled membrane transport protein;
- 5 (b) (i) permeabilizing or disrupting the plasma membrane of a cell and contacting the labeled membrane transport protein within the cell with the ligand of the label for a time and under conditions sufficient for the ligand to bind the label; and
- (ii) determining the level of ligand bound to the labeled membrane transport protein within the cell; and
- 10 (c) comparing the level of ligand detected at (a) (ii) and (b) (ii) to determine the level of the labeled membrane transport protein at the plasma membrane relative to the level of the labeled membrane transport protein inside the cell.

- 15 For example, the label is a peptide, polypeptide or protein that is heterologous to the membrane transport protein. Such a label facilitates detection of the membrane transport protein with which the peptide, polypeptide or protein is associated.

A suitable detectable label includes, for example, a peptide, polypeptide or protein to which an antibody or ligand is capable of specifically binding. Alternatively, or in addition, the label is, for example, an enzyme that catalyzes a detectable reaction when contacted with a suitable substrate.

An example of a suitable detectable peptide polypeptide or protein is selected from the group consisting of influenza virus hemagglutinin (HA) (SEQ ID NO: 15), Simian Virus 5 (V5) (SEQ ID NO: 16), polyhistidine (SEQ ID NO: 17), c-myc (SEQ ID NO: 18), FLAG (SEQ ID NO: 19), an epitope tag described by Sloostra *et al.*, *Mol. Drivers* 2: 156 - 164 (SEQ ID NO: 20 or SEQ ID NO: 21), GST (SEQ ID NO: 22), MBP (SEQ ID NO: 23), GAL4 (SEQ ID NO: 24), β -galactosidase (SEQ ID NO: 25), enhanced green fluorescence protein (eGFP) (SEQ ID NO: 26), yellow fluorescent protein (SEQ ID NO: 27), soluble modified blue fluorescent protein (SEQ ID NO: 28), soluble-modified red-shifted green fluorescent protein (SEQ ID NO: 29) and cyan fluorescent protein (SEQ ID NO: 30).

- 35 Alternatively, the membrane transport protein is labeled with a protein that directly associates with another known protein, such as for example, biotin, streptavidin or the

Strep-Tag, an 8 amino acid strepavidin binding sequence (WSHPQFEK, SEQ ID NO: 31) (available from Sigma-Genosys, Sydney, Australia).

In an exemplified embodiment of the invention, the label that is linked to a membrane transport protein is a HA tag (SEQ ID NO: 15).

In one form of the invention, the label is linked or fused to an extracellular domain of a membrane transport protein. Accordingly, it is preferable that the labeled membrane transport protein is a fusion protein. As used herein, the term "extracellular domain" shall be taken to mean the region or component of a protein that is located external to the cell when the membrane transport protein is incorporated into the plasma membrane. Accordingly, when a membrane transport protein is not incorporated into the plasma membrane of a cell, the extracellular domain may be located within the cell.

Methods for determining the subcellular localization of a domain of a protein are known in the art. For example the following programs are useful for determining an extracellular domain of a protein:

- i) PSORT, based on Horton and Nakai *Proc Int Conf Intell Syst Mol Biol.*;5:147-52, 1997) is available from the Brinkman Laboratory at Simon Fraser University, Burnaby, British Columbia, Canada;
- ii) TopPred 2 based on Gunnar von Heijne, *J. Mol. Biol.* 225, 487-494, 1992 available from Stockholm University;
- iii) HMMTOP based on Tusnády and Simon *J. Mol. Biol.* 283: 489-506, 1998 available from The Institute of Enzymology, Hungarian Academy of Sciences, Budapest; and
- iv) SOSUI available from Department of Biotechnology, Tokyo University of Agriculture and Technology.

Alternatively, or in addition, a region of a membrane transport protein that is extracellular is predicted using the method described, for example, in Nakashima and Nishikawa, *FEBS Lett.* 303: 141-146, 1992; Nakashima and Nishikawa, *J. Mol. Biol.*, 238: 54-61, 1994; Rost *et al*, *Prot Sci.*, 4: 521-533, 1995; or Chou and Cai, *Biochem Biophys Res Commun.* 320:1236-9, 2004. Such methods rely upon the analysis of the amino acid composition of a membrane transport protein to determine, for example, hydropathy of regions of the protein to determine a region that is extracellular or intracellular.

In an exemplified form of the invention, the tag is linked or fused to the first exofacial or extracellular loop of the GLUT4 protein or a mutant thereof. For example, This protein comprises the sequence set forth in SEQ ID NO: 4 and/or is encoded by a nucleic acid set forth in SEQ ID NO: 3. A labeled TAIL mutant of GLUT4 comprises, for example, the sequence set forth in SEQ ID NO: 6. A labeled L489, 490A mutant of GLUT4 comprises, for example, the sequence set forth in SEQ ID NO: 8. A labeled F5A mutant of GLUT4 comprises, for example, the sequence set forth in SEQ ID NO: 10.

In an example of the invention, the label is covalently linked to the membrane transport protein. For example, a disulfide bond is formed between the label and the membrane transport protein. As will be apparent to the person skilled in the art such a membrane transport protein is then be delivered to the cell. In one embodiment the peptide encoded by the nucleic acid fragment of the present invention is expressed as a fusion protein with a peptide sequence capable of enhancing, increasing or assisting penetration or uptake of the protein by cells. Means and methods of enhancing, increasing or assisting penetration or uptake of the membrane transport protein by cells are described, for example, In Morris *et al*, *Nature Biotechnology* 19, 1173-1176, 2001.

In an alternative example, the membrane transport protein is expressed as a fusion protein with the label (e.g., as a recombinant fusion protein). As will be apparent to the skilled artisan, a fusion protein is advantageously expressed within a cell using an expression construct. As used herein, the term "expression construct" is to be taken in its broadest context and includes a promoter sequence that is placed in operable connection with a nucleic acid that encodes a membrane transport protein (e.g., a labeled membrane transport protein) of the present invention.

The term "promoter" is to be taken in its broadest context and includes the transcriptional regulatory sequences of a genomic gene, including the TATA box or initiator element, which is required for accurate transcription initiation, with or without additional regulatory elements (i.e. upstream activating sequences, transcription factor binding sites, enhancers and silencers) which alter gene expression in response to developmental and/or external stimuli, or in a tissue specific manner. In the present context, the term "promoter" is also used to describe a recombinant, synthetic or fusion molecule, or derivative which confers, activates or enhances the expression of a nucleic

acid molecule to which it is operably linked, and which encodes the peptide or protein. Preferred promoters can contain additional copies of one or more specific regulatory elements to further enhance expression and/or alter the spatial expression and/or temporal expression of said nucleic acid molecule.

5

Placing a nucleic acid molecule under the regulatory control of, i.e., "in operable connection with", a promoter sequence means positioning said molecule such that expression is controlled by the promoter sequence. Promoters are generally positioned 5' (upstream) to the coding sequence that they control. To construct heterologous

- 10 promoter/structural gene combinations, it is generally preferred to position the promoter at a distance from the gene transcription start site that is approximately the same as the distance between that promoter and the gene it controls in its natural setting, i.e., the gene from which the promoter is derived. As is known in the art, some variation in this distance can be accommodated without loss of promoter function.
- 15 Similarly, the preferred positioning of a regulatory sequence element with respect to a heterologous gene to be placed under its control is defined by the positioning of the element in its natural setting, i.e., the gene from which it is derived. Again, as is known in the art, some variation in this distance can also occur.

- 20 Typical promoters suitable for expression in a virus of a mammalian cell, or in a mammalian cell, mammalian tissue or intact mammal include, for example a promoter selected from the group consisting of, a retroviral LTR element, a SV40 early promoter, a SV40 late promoter, a cytomegalovirus (CMV) promoter, a CMV IE (cytomegalovirus immediate early) promoter, an EF_{1 α} promoter (from human
- 25 elongation factor 1 α), an EM7 promoter or an UbC promoter (from human ubiquitin C).

- Typical promoters suitable for expression in viruses of bacterial cells and bacterial cells such as for example a bacterial cell selected from the group comprising *E. coli*,
- 30 *Staphylococcus* sp, *Corynebacterium* sp., *Salmonella* sp., *Bacillus* sp., and *Pseudomonas* sp., include, but are not limited to, the *lacZ* promoter, the Ipp promoter, temperature-sensitive λ_L or λ_R promoters, T7 promoter, T3 promoter, SP6 promoter or semi-artificial promoters such as the IPTG-inducible *tac* promoter or lacUV5 promoter. A number of other gene construct systems for expressing the nucleic acid fragment of
- 35 the invention in bacterial cells are well-known in the art and are described for example, in Ausubel *et al* (In: Current Protocols in Molecular Biology. Wiley Interscience, ISBN

047 150338, 1987) and (Sambrook *et al* (In: Molecular Cloning: Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratories, New York, Third Edition 2001).

Typical promoters suitable for expression in yeast cells such as for example a yeast cell
5 selected from the group comprising *Pichia pastoris*, *S. cerevisiae* and *S. pombe*, include, but are not limited to, the *ADHI* promoter, the *GALI* promoter, the *GAL4* promoter, the *CUP1* promoter, the *PHO5* promoter, the *nmt* promoter, the *RPR1* promoter, or the *TEF1* promoter.

10 Methods for producing expression constructs are known in the art and are described, for example, in Ausubel *et al* (In: Current Protocols in Molecular Biology. Wiley Interscience, ISBN 047 150338, 1987) or Sambrook *et al* (In: Molecular Cloning: Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratories, New York, Third Edition 2001).

15

In one embodiment, the expression construct forms a component of an expression vector. The term "expression vector" refers to a nucleic acid molecule that has the ability to confer expression on a nucleic acid to which it is operably connected, in a cell or in a cell free expression system. Within the context of the present invention, it is to
20 be understood that an expression vector may comprise a promoter as defined herein, a plasmid, bacteriophage, phagemid, cosmid, virus sub-genomic or genomic fragment, or other nucleic acid capable of maintaining and or replicating heterologous DNA in an expressible format. Many expression vectors are commercially available for expression in a variety of cells. Selection of appropriate vectors is within the knowledge of those
25 having skill in the art.

For example, expression vectors that contain suitable promoter sequences for expression in mammalian cells or mammals include, but are not limited to, the pcDNA vector suite supplied by Invitrogen, the pCI vector suite (Promega), the pCMV vector
30 suite (Clontech), the pM vector (Clontech), the pSI vector (Promega) or the VP16 vector (Clontech).

Numerous expression vectors for expression of recombinant polypeptides in bacterial cells and efficient ribosome binding sites have been described, such as for example,
35 PKC30 (Shimatake and Rosenberg, *Nature* 292, 128, 1981); pKK173-3 (Amann and Brosius, *Gene* 40, 183, 1985), pET-3 (Studier and Moffat, *J. Mol. Biol.* 189, 113,

1986); the pCR vector suite (Invitrogen), pGEM-T Easy vectors (Promega), the pL expression vector suite (Invitrogen) the pBAD/TOPO (Invitrogen, Carlsbad, CA); the pFLEX series of expression vectors (Pfizer Inc., CT,USA); the pQE series of expression vectors (QIAGEN, CA, USA), or the pL series of expression vectors
5 (Invitrogen), amongst others.

Expression vectors for expression in yeast cells are known in the art and include, but are not limited to, the pACT vector (Clontech), the pDBleu-X vector, the pPIC vector suite (Invitrogen), the pGAPZ vector suite (Invitrogen), the pHYB vector (Invitrogen), the
10 pYD1 vector (Invitrogen), and the pNMT1, pNMT41, pNMT81 TOPO vectors (Invitrogen), the pPC86-Y vector (Invitrogen), the pRH series of vectors (Invitrogen), pYESTrp series of vectors (Invitrogen).

Following production of a suitable gene construct, said construct is introduced into the
15 relevant cell. Methods of introducing the gene constructs into a cell or organism for expression are well known to those skilled in the art and are described for example, in Ausubel *et al* (In: Current Protocols in Molecular Biology. Wiley Interscience, ISBN 047 150338, 1987) and (Sambrook *et al* (In: Molecular Cloning: Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratories, New York, Third Edition 2001).
20 The method chosen to introduce the gene construct in depends upon the cell type in which the gene construct is to be expressed. Means for introducing recombinant DNA into bacterial cells include, but are not limited to electroporation or chemical transformation into cells previously treated to allow for said transformation, PEG mediated transformation, microinjection, transfection mediated by DEAE-dextran,
25 transfection mediated by calcium phosphate, transfection mediated by liposomes such as by using Lipofectamine (Invitrogen) and/or cellfectin (Invitrogen), transduction by Adenoviruses, Herpesviruses, Togaviruses or Retroviruses and microparticle bombardment such as by using DNA-coated tungsten or gold particles (Agacetus Inc., WI, USA).

30

As exemplified herein, the present inventors have used a retroviral system to transfect or transduce a cell with an expression construct encoding a membrane transport protein. Accordingly, a viral delivery system is contemplated by the present invention.

35 Conventional viral based systems for the delivery of a nucleic acid include, for example, retroviral, lentivirus, adenoviral, adeno-associated virus and herpes simplex

virus. Viral vectors are an efficient and versatile method of gene transfer in target cells and tissues. Integration in the host cell genome occurs with the retrovirus, lentivirus, and adeno-associated virus gene transfer methods, often resulting in long term expression of the inserted expression construct. Additionally, high transduction efficiencies have been observed in many different cell types and target tissues.

The tropism of a retrovirus can be altered by incorporating foreign envelope proteins, expanding the potential target population of target cells. A lentiviral vector is a retroviral vector that is capable of transducing or infecting a non-dividing cell and typically produces high viral titers. Selection of a retroviral gene transfer system depends on the target tissue.

A Retroviral vector comprises cis-acting long terminal repeats (LTRs) with packaging capacity for up to 6-10 kb of foreign sequence. The minimum cis-acting LTRs are sufficient for replication and packaging of the vectors, which are then used to integrate the membrane transport gene into the target cell to provide long term transgene expression. Widely used retroviral vectors include those based upon murine leukemia virus (MuLV), gibbon ape leukemia virus (GaLV), simian immunodeficiency virus (SIV), human immunodeficiency virus (HIV), and combinations thereof (see, e.g., Buchscher et al., *J. Virol.* 66:2731-2739 (1992); Johann et al., *J. Virol.* 66:1635-1640 (1992); Sommerfelt et al., *Virol.* 176:58-59 (1990); Wilson et al., *J. Virol.* 63:274-2378 (1989); Miller et al., *J. Virol.* 65:2220-2224 (1991); PCT/US94/05700; Miller and Rosman *BioTechniques* 7:980-990, 1989; Miller, A. D. *Human Gene Therapy* 1:5-14, 1990; Scarpa et al) *Virology* 180:849-852, 1991; Burns et al. *Proc. Natl. Acad. Sci. USA* 90:8033-8037, 1993.).

In applications where transient expression of the nucleic acid is preferred, adenoviral based systems are typically used. Adenoviral based vectors are capable of high transduction efficiency in many cell types and do not require cell division. With such vectors, high titer and levels of expression have been obtained. This vector can be produced in large quantities in a relatively simple system. (see, e.g., West et al., *Virology* 160:38-47 1987; U.S. Pat. No. 4,797,368; WO 93/24641; Kotin, *Human Gene Therapy* 5:793-801 1994; Muzyczka. *Clin. Invest.* 94:1351 1994).

Various adeno-associated virus (AAV) vector systems have also been developed for nucleic acid delivery. AAV vectors can be readily constructed using techniques known

in the art. See, e.g., U.S. Pat. Nos. 5,173,414 and 5,139,941; International Publication Nos. WO 92/01070 and WO 93/03769; Lebkowski *et al.* *Molec. Cell. Biol.* 8:3988-3996, 1988; Vincent *et al.* (1990) *Vaccines 90* (Cold Spring Harbor Laboratory Press); Carter *Current Opinion in Biotechnology* 3:533-539, 1992; Muzyczka. *Current Topics* 5 *in Microbiol. and Immunol.* 158:97-129, 1992; Kotin, *Human Gene Therapy* 5:793-801, 1994; Shelling and Smith *Gene Therapy* 1:165-169, 1994; and Zhou *et al.* *J. Exp. Med.* 179:1867-1875, 1994.

Additional viral vectors useful for delivering a nucleic acid encoding membrane transport protein by gene transfer include those derived from the pox family of viruses, such as vaccinia virus and avian poxvirus or an alphavirus or a conjugate virus vector (e.g. that described in Fisher-Hoch *et al.*, *Proc. Natl. Acad. Sci. USA* 86:317-321, 1989).

As will be apparent from the preceding description, the present invention also encompasses providing the cell that expresses a membrane protein. The term "providing the cell that expresses a membrane protein" shall be taken to include transforming, transfecting or transducing a cell with an expression construct that encodes the membrane transport protein. Optionally, the term "providing the cell that expresses a membrane protein" shall be taken to additionally mean preparing the expression construct that encodes the membrane transport protein.

Suitable cells

As membrane transfer proteins are found in the majority of species any cell that expresses a membrane transport protein in nature is suitable for the performance of the instant invention. For example, transporters, channels and primary active transporters are found in bacterium, yeast, plants and mammals, see, for example, Chung *et al.*, *Journal of Bacteriology*, 183: 1012-1021, 2001. Furthermore, ABC transport proteins are found in bacterium, yeast and mammals.

In an example of the invention, the cell is a eukaryotic cell, for example, a mammalian cell.

As will be apparent to the skilled artisan, the process of the present invention is preferably performed *in vitro*. Accordingly, the invention is performed, for example, using a cell isolated from a subject or using a cell line.

In one example of the invention, the method is performed in a cell that is amenable to transformation, transfection or transduction. For example, the cell is a cell selected from the group consisting of COS, CHO, murine 10T, MEF, NIH3T3, MDA-MB-231,
5 MDCK, HeLa, K562, HEK 293, 3T3-L1 and 293T.

COS cells have been previously shown to be amenable to both transfection/transduction and the study of translocation of a membrane transport protein, particularly a GLUT4 protein.

10

In another example, a cell useful for performance of the process of the invention is a cell that is known to express and/or translocate the membrane transport protein of interest in nature. For example, muscle cells and adipocyte cells are known to express and translocate GLUT4 in nature. Accordingly, a muscle cell selected from the group
15 consisting of a C2C12 cell, a L8 cell, a L6 cell, a F3 cell, a 10T1/2 cell, a H9C2 cell and a BC3H cell is useful for the performance of the invention. Alternatively, or in addition, an adipocyte cell or a pre-adipocyte cell selected from the group consisting of a 3T3-L1 cell, a HIB1B cell and a PA26 cell is useful for the performance of the invention.

20

As GLUT1 is also expressed and translocated in a muscle cell the muscle cells described *supra* are useful for the performance of the process of the invention to assess the translocation of GLUT4.

25 The translocation of CFTR is, for example, studied in a cell line derived from a tissue affected in cystic fibrosis, e.g., a Calu-3 airway epithelium cell line or a T84 colonic cell line.

Alternatively, the translocation of a membrane transport protein is studied using a
30 primary cell, i.e. a cell isolated from a subject. For example, methods of isolating an adipocyte, a pre-adipocyte, a fibroblast, a muscle cell or an airway epithelium cell are known in the art. For example, Katoh *et al.*, *Folia Histochem Cytobiol.* 32:235-8, 1994 describe a method for isolating a pre-adipocyte cell from adipose tissue.

35 *Detection of a membrane transport protein*

To determine the level of a membrane transport protein at the plasma membrane of a cell, a ligand is selected that is capable of specifically binding the membrane transport, for example, a ligand capable of binding to the label of a labeled membrane transport protein.

5

As used herein the term "ligand" shall be taken in its broadest context to include any chemical compound, polynucleotide, peptide, protein, lipid, carbohydrate, small molecule, natural product, polymer, etc. that is capable of selectively binding, whether covalently or not, to one or more specific sites on a target molecule, e.g., a labeled
10 membrane transport protein (e.g., a label associated with or bound to the membrane transport protein). The ligand may bind to its target via any means including hydrophobic interactions, hydrogen bonding, electrostatic interactions, van der Waals interactions, pi stacking, covalent bonding, or magnetic interactions amongst others.

15 In one example of the invention, the ligand is an antibody. As used herein the term "antibody" refers to intact monoclonal or polyclonal antibodies, immunoglobulin (IgA, IgD, IgG, IgM, IgE) fractions, humanized antibodies, or recombinant single chain antibodies, as well as fragments thereof, such as, for example Fab, F(ab)₂, and Fv fragments.

20

Antibodies referred to herein are obtained from a commercial source, or alternatively, produced by conventional means. Commercial sources will be known to those skilled in the art. For example, Sigma-Aldrich (Sydney, Australia) sell monoclonal antibodies that specifically bind HA, FLAG, V5, polyhistidine, c-myc, GST, MBP, β -
25 galactosidase, GFP or biotin. The present inventors have used an anti-HA monoclonal antibody to determine the level of translocation of a HA tagged membrane transport protein (eg., a HA-tagged GLUT4 protein).

High titer antibodies are preferred, as these are more useful commercially in kits for
30 analytical, diagnostic and/or therapeutic applications. By "high titer" is meant a titer of at least about $1:10^3$ or $1:10^4$ or $1:10^5$. Methods of determining the titer of an antibody will be apparent to the skilled artisan. For example, the titer of an antibody in purified antiserum may be determined using an ELISA assay to determine the amount of IgG in a sample. Typically an anti-IgG antibody or Protein G is used in such an assay. The
35 amount detected in a sample is compared to a control sample of a known amount of

purified and/or recombinant IgG. Alternatively, a kit for determining antibody may be used, e.g. the Easy TITER kit from Pierce (Rockford, IL, USA).

Antibodies may be prepared by any of a variety of techniques known to those of ordinary skill in the art, and are described, for example in, Harlow and Lane (*In: Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, 1988). In one such technique, an immunogen comprising the antigenic polypeptide is initially injected into any one of a wide variety of animals (e.g., mice, rats, rabbits, sheep, humans, dogs, pigs, chickens and goats). The immunogen is derived from a natural source, produced by recombinant expression means, or artificially generated, such as by chemical synthesis (e.g., BOC chemistry or Fmoc chemistry).

A peptide, polypeptide or protein is optionally joined to a carrier protein, such as bovine serum albumin or keyhole limpet hemocyanin. The immunogen and optionally a carrier for the protein is injected into the animal host, preferably according to a predetermined schedule incorporating one or more booster immunizations, and blood collected from said the animals periodically. Optionally the immunogen is injected in the presence of an adjuvant, such as, for example Freund's complete or incomplete adjuvant, lysolecithin and/or dinitrophenol to enhance the immune response to the immunogen. Monoclonal or polyclonal antibodies specific for the polypeptide are then be purified from the blood isolated from an animal by, for example, affinity chromatography using the polypeptide coupled to a suitable solid support.

Monoclonal antibodies specific for the antigenic polypeptide of interest may be prepared, for example, using the technique of Kohler and Milstein, *Eur. J. Immunol.* 6:511-519, 1976, and improvements thereto. Briefly, these methods involve the preparation of immortal cell lines capable of producing antibodies having the desired specificity (i.e., reactivity with the polypeptide of interest). Such cell lines may be produced, for example, from spleen cells obtained from an animal immunized as described *supra*. The spleen cells are then immortalized by, for example, fusion with a myeloma cell fusion partner, preferably one that is syngenic with the immunized animal. A variety of fusion techniques may be employed, for example, the spleen cells and myeloma cells may be combined with a nonionic detergent or electrofused and then grown in a selective medium that supports the growth of hybrid cells, but not myeloma cells. A preferred selection technique uses HAT (hypoxanthine, aminopterin, thymidine) selection. After a sufficient time, usually about 1 to 2 weeks, colonies of

hybrids are observed. Single colonies are selected and growth media in which the cells have been grown is tested for the presence of binding activity against the polypeptide (immunogen). Hybridomas having high reactivity and specificity are preferred.

- 5 Monoclonal antibodies are isolated from the supernatants of growing hybridoma colonies using methods such as, for example, affinity purification as described *supra*. In addition, various techniques may be employed to enhance the yield, such as injection of the hybridoma cell line into the peritoneal cavity of a suitable vertebrate host, such as a mouse. Monoclonal antibodies are then harvested from the ascites fluid or the
10 blood of such an animal subject. Contaminants are removed from the antibodies by conventional techniques, such as chromatography, gel filtration, precipitation, and/or extraction.

- It is preferable that an immunogen used in the production of an antibody is one which
15 is sufficiently antigenic to stimulate the production of antibodies that will bind to the immunogen and is preferably, a high titer antibody. For example, an immunogen may be an entire protein.

- Alternatively, an immunogen consists of a peptide representing a fragment of a
20 polypeptide. Preferably, an antibody raised to such an immunogen also recognizes the full-length protein from which the immunogen was derived, such as, for example, in its native state or having native conformation.

- As discussed *supra* antibody fragments are contemplated by the present invention. The
25 term "antibody fragment" refers to a portion of a full-length antibody, generally the antigen binding or variable region. Examples of antibody fragments include Fab, Fab', F(ab')₂ and Fv fragments.

- Papain digestion of an antibody produces two identical antigen binding fragments,
30 called the Fab fragment, each with a single antigen binding site, and a residual "Fc" fragment.

- Pepsin treatment yields an F(ab')₂ fragment that has two antigen binding fragments that are capable of cross-linking antigen, and a residual other fragment (which is termed
35 pFc'). Additional fragments can include diabodies, linear antibodies, single-chain antibody molecules, and multispecific antibodies formed from antibody fragments. As

used herein, "functional fragment" with respect to antibodies, refers to Fv, F(ab) and F(ab')₂ fragments.

An "Fv" fragment is the minimum antibody fragment that contains a complete antigen
5 recognition and binding site. This region consists of a dimer of one heavy and one light
chain variable domain in a non-covalent association (V_H -V_L dimer). It is in this
configuration that the three CDRs of each variable domain interact to define an antigen
binding site on the surface of the V_H -V_L dimer. Collectively, the six CDRs confer
10 antigen binding specificity to the antibody. However, even a single variable domain (or
half of an Fv comprising only three CDRs specific for an antigen) has the ability to
recognize and bind antigen.

A Fab fragment [also designated as F(ab)] also contains the constant domain of the
light chain and the first constant domain (CH1) of the heavy chain. Fab' fragments
15 differ from Fab fragments by the addition of a few residues at the carboxyl terminus of
the heavy chain CH1 domain including one or more cysteines from the antibody hinge
region. F(ab') fragments are produced by cleavage of the disulfide bond at the hinge
cysteines of the F(ab')₂ pepsin digestion product. Additional chemical couplings of
antibody fragments are known to those of ordinary skill in the art.

20 "Single-chain Fv" or "scFv" antibody fragments comprise the V_H and V_L domains of
an antibody, wherein these domains are present in a single polypeptide chain.
Generally, the Fv polypeptide further comprises a polypeptide linker between the V_H
and V_L domains which enables the scFv to form the desired structure for antigen
25 binding. For a review of scFv, see Pluckthun in *The Pharmacology of Monoclonal
Antibodies*, vol. 113, Rosenberg and Moore eds. Springer-Verlag, New York, pp. 269-
315 (1994).

30 In another example, a ligand is a small molecule. Chemical small molecule libraries
are available commercially or alternatively may be generated using methods known in
the art, such as, for example, those described in U.S. Patent No. 5,463,564.

Alternatively, a ligand is a peptidyl ligand. A peptidyl ligand are conveniently made by
standard peptide synthesis, such as the Merrifield method of synthesis (Merrifield, *J*
35 *Am Chem Soc*, 85,2149-2154, 1963) and the myriad of available improvements on that
technology (see e.g., *Synthetic Peptides: A User's Guide*, Grant, ed. (1992) W.H.

Freeman & Co., New York, pp. 382; Jones (1994) *The Chemical Synthesis of Peptides*, Clarendon Press, Oxford, pp. 230.).

For example, a membrane transport protein is labeled with strepavidin and the peptidyl
5 ligand is a peptide that comprises a strepavidin binding sequence, e.g. the amino acid sequence set forth in SEQ ID NO: 31.

Alternatively, the membrane transport protein is labeled with biotin and the ligand is strepavidin.

10

As will be apparent to the skilled artisan, a preferred ligand is not capable of independently entering a cell that has not been permeabilized or disrupted. Accordingly, when a cell with an intact plasma membrane is contacted with the ligand, said ligand will bind to the membrane transport protein in the plasma membrane, and
15 not to the membrane protein within the cell to a significant degree.

However, the present inventors have shown that the ligand may be capable of entering the cell when bound to a membrane transport protein that recycles away from the membrane without significantly altering the efficacy of the test. In fact, such a ligand
20 is useful for determining internalization and/or a rate of internalization of a membrane transport protein.

A ligand useful in the process of the present invention is, for example, labeled with a detectable marker. For example, a fluorescent label (e.g. FITC or Texas Red), a
25 fluorescent semiconductor nanocrystal (as described in US 6,306,610), a radiolabel or an enzyme (e.g. horseradish peroxidase (HRP), alkaline phosphatase (AP) or β -galactosidase)

An example of a suitable fluorescent label include fluorescein (FITC), 5,6-carboxymethyl fluorescein, Texas red, nitrobenz-2-oxa-1,3-diazol-4-yl (NBD), coumarin, dansyl chloride, rhodamine, 4'-6-diamidino-2-phenylindole (DAPI), and the cyanine dyes Cy3, Cy3.5, Cy5, Cy5.5 and Cy7, fluorescein (5-carboxyfluorescein-N-hydroxysuccinimide ester), rhodamine (5,6-tetramethyl rhodamine). The absorption and emission maxima, respectively, for these fluors are: FITC (490 nm; 520 nm), Cy3
30 (554 nm; 568 nm), Cy3.5 (581 nm; 588 nm), Cy5 (652 nm; 672 nm), Cy5.5 (682 nm; 703 nm) and Cy7 (755 nm; 778 nm).

In an exemplified form of the invention a suitable fluorescent label is, for example, a fluorescent label obtained from Molecular Probes, Eugene, OR, such as, for example Alexafluor®350, Alexafluor® 488, Alexafluor® 555, Alexafluor® 594 or Alexafluor®
5 647. Such an antibody may be purchased from a commercial source. Alternatively, Molecular Probes supplies kits for labeling an antibody or proteinaceous ligand with such a fluorescent label.

In another example, the label is a fluorescent nanocrystal. A fluorescent nanocrystal
10 generally comprises a core composed of cadmium sulfide (CdS), cadmium selenide (CdSe), or cadmium telluride (CdTe). The size and shape of the core aids in determining the wavelength at which the nanocrystal fluoresce. Coating the core is a shell composed of a non-emissive transparent but structurally related material, for example, ZnS. Finally, such a fluorescent nanocrystal is coated to provide a
15 carboxylate surface to which many biological and nonbiological moieties may be attached. Such a nanocrystal is then conjugated to a ligand of interest, eg., an antibody, for example using an antibody conjugation kit from Qdot® (Hayward, CA). By exciting the nanocrystal at the relevant wavelength, the crystal emits a fluorescent light that is detectable using a method known in the art and/or described herein.

20 In a further example, the label is an enzymatic label. For example, a ligand is conjugated to β -galactosidase. Following contacting the cell and/or membrane transport protein with such a ligand, the sample is contacted with, for example, 5-bromo-4-chloro-3-indol-beta-D-galaotopyranoside (x-gal). The resulting reaction
25 causes a blue colored precipitate to form. Other enzymatic labels are known in the art and include, for example, alkaline phosphatase or horseradish peroxidase (HRP). Suitable substrates for such enzymes are known in the art and include, for example, hydrogen peroxide or 3-3,5,5'-tetramethylbenzidine (TMB).

30 In another example, the ligand that binds to the label is detected using another ligand, such as, for example, an antibody. For example the secondary antibody/ligand is capable of specifically binding to the ligand that binds to the label. The present inventors have used a mouse monoclonal antibody to bind a labeled membrane transport protein and an anti-mouse secondary antibody to detect binding of the mouse
35 monoclonal antibody. Preferably, the secondary antibody is labeled with a detectable marker, such as, for example, a marker described *supra*.

Alternatively, a ligand that binds to a label or a secondary antibody/ligand is conjugated to, for example, biotin. Streptavidin is capable of binding to biotin with high affinity and specificity. Accordingly, streptavidin labeled with a detectable marker is
5 useful for detecting the binding of the ligand that binds to a label or a secondary antibody/ligand. A suitable detectable marker will be apparent to the skilled artisan, for example, a marker described *supra*.

Detection methods

- 10 Methods for detecting the binding of the ligand to the label and/or the secondary antibody/ligand to the primary ligand are known in the art and/or described herein. For example, such detection methods are described in Scopes (*In: Protein purification: principles and practice*, Third Edition, Springer Verlag, 1994).
- 15 In one form of the invention, the level of the ligand bound to the membrane transport protein is determined by a process comprising contacting the ligand with an antibody that specifically binds the label for a time and under conditions sufficient for the antibody to bind and determining the level of bound antibody.
- 20 As will be apparent to the skilled artisan, the detection method used depends upon the type of label used.

For example, a standard solid-phase ELISA format is useful in determining the level of an enzyme labeled ligand or antibody.

25

In one form such an assay involves immobilizing or growing or incubating the cell *supra* onto a solid matrix, such as, for example a polystyrene or polycarbonate microwell or dipstick, a membrane, or a glass support (e.g. a glass slide). Preferably, the ELISA assay is performed upon the plate upon which the cells are grown.

30

- An antibody or ligand that specifically binds the membrane transport protein or label is brought into direct contact with the cell, and forms a direct bond with any of the membrane transport protein or label present in said sample. This antibody is generally labeled with a detectable reporter molecule, such as for example, an enzyme (e.g.
35 horseradish peroxidase (HRP)), alkaline phosphatase (AP) or β -galactosidase. Alternatively, a second labeled antibody can be used that binds to the first antibody.

Following washing to remove any unbound antibody the detectable marker is detected by the addition of a substrate, such as for example hydrogen peroxide, TMB, or toluidine, or 5-bromo-4-chloro-3-indol-beta-D-galaotopyranoside (x-gal).

- 5 The level of the membrane transport protein may be determined using a standard curve that has been produced using known quantities of the membrane transport protein (e.g. recombinant membrane transport protein).

In the case of a fluorescent label, a fluorescence linked immunosorbent assay (FLISA)
10 is useful for determining the level of a labeled ligand or antibody in a sample. A FLISA is performed essentially as described *supra* for the ELISA assay, however, a substrate is not required to detect the bound labeled ligand or antibody. Rather, following washing to remove any unbound ligand/antibody the sample is exposed to a light source of the appropriate wavelength and the level of fluorescence emitted by
15 each sample determined. A FLISA is also known as an immunofluorescence assay (IFA). The present inventors have clearly exemplified this form of assay.

As will be apparent to the skilled artisan, other detection methods based on an immunosorbent assay are useful in the performance of the present invention. For
20 example, an immunosorbent method based on the description *supra* using a radiolabel for detection, or a gold label (e.g. colloidal gold) for detection, or a liposome, for example, encapsulating NAD⁺ for detection (e.g., as described in Kumada *et al.*, *Journal of Chemical Engineering of Japan*, 34: 943-947, 2001) or an acridinium linked immunosorbent assay.

25 In another example, the level of the labeled ligand or antibody is determined using immunohistochemistry and/or immunofluorescence. For example, a cell or tissue section that is to be analyzed is optionally fixed to stabilize and protect both the cell and the proteins contained within the cell. Preferably, the method of fixation does not
30 disrupt or destroy the antigenicity of the membrane transport protein, thus rendering it undetectable. Methods for fixing a cell are known in the art and include for example, treatment with paraformaldehyde, treatment with alcohol, treatment with acetone, treatment with methanol, treatment with Bouin's fixative and treatment with glutaraldehyde. Following fixation a cell is incubated with a ligand or antibody
35 capable of binding the membrane transport protein. As discussed *supra* the ligand or antibody may be labeled with a detectable marker. Alternatively, a second labeled

antibody that binds to the first antibody can be used to detect the first antibody. Following washing to remove any unbound antibody, the level of ligand or antibody bound to the membrane transport protein is determined using an appropriate means. Means for detecting a label vary depending upon the type of label used and will be
5 apparent to the skilled artisan.

Methods using immunofluorescence are preferable, as they are quantitative or at least semi-quantitative. Methods of quantitating the degree of fluorescence of a stained cell are known in the art and described, for example, in Immunohistochemistry (Cuello,
10 1984 John Wiley and Sons, ASIN 0471900524).

A high-throughput method of immunohistochemical/immunofluorescent analysis of a biological sample are preferred. For example, the EIDAQ 100 - HTM system of Q3DM (San Diego, CA, USA) allows the rapid automatic analysis of a biological
15 sample to determine the presence and/or level of a polypeptide of interest.

Determining the level of a membrane transport protein within a sample

Following determining the level of membrane transport protein that has translocated to the plasma membrane of a cell, the total amount of that membrane transport protein in
20 the cell is determined using a method known in the art and/or described herein.

Accordingly, comparison of the level of the membrane transport protein that has translocated to the plasma membrane to the level of the membrane transport protein detected in the cell provides a relative estimate of the level of the membrane transport
25 protein that has translocated to the plasma membrane as a function of total membrane transport protein (for example as a percentage of total membrane transport protein). Such an estimate effectively "normalizes" the results of such an assay, reducing inter-assay variability and allowing comparisons between multiple assays.

30 To determine the total amount of membrane transport protein in a cell, the plasma membrane is permeabilized or disrupted to allow the detection means, e.g. a ligand or antibody, to enter the cell and bind the membrane transport protein. In permeabilizing or disrupting a cell membrane it is important that the membrane transport protein within the cell is not significantly degraded.

35 Methods for permeabilizing a cell are known in the art and/or described herein.

For example, a cell or plasma membrane is contacted with an agent or compound that permeabilizes or disrupts a membrane for a time and under conditions sufficient for permeabilization or disruption to occur.

5

A suitable agent or compound that permeabilizes or disrupts a plasma membrane will be apparent to the skilled artisan. For example, a suitable agent or compound that permeabilizes or disrupts a plasma membrane is selected from the group consisting of saponin, n-octyl-glucopyranoside, n-Dodecyl β -D-maltoside, N-Dodecanoyl-N-methylglycine sodium salt, hexadecyltrimethylammonium bromide, deoxycholate, a
10 non-ionic detergent, streptolysin-O (SEQ ID NO: 32), α -hemolysin (SEQ ID NO: 33), tetanolysin (SEQ ID NO: 34) and mixtures thereof.

Agents useful for disrupting or permeabilizing a membrane are commercially available
15 from, for example, Sigma-Aldrich, Sydney, Australia. For example, saponin, n-octyl-glucopyranoside, n-Dodecyl β -D-maltoside, hexadecyltrimethylammonium bromide, streptolysin-O, α -hemolysin or tetanolysin are commercially available from Sigma Aldrich.

20 The present inventors contacted a cell with a suitable amount of saponin for a time and under conditions suitable to disrupt or permeabilize a plasma membrane. This method permeabilized the plasma membrane sufficiently to facilitate detection of the level of membrane transport protein within the cell.

25 Methods for using other agents for permeabilizing a plasma membrane will be apparent to the skilled artisan. For example, Palmer *et al.*, *EMBO J.* 17: 1598-1605, 1998 describe the use of Streptolysin-O to disrupt or permeabilize the membrane of a cell. Gariglio *FEBS Lett.* 44, 330, 1974, described the use of N-Dodecanoyl-N-methylglycine sodium salt for the lysis of eukaryotic cells.

30

In an example of the invention a cell is fixed. Methods for fixing a cell are known in the art and/or described herein. In one example, the cell is fixed using a process comprising contacting a cell with a fixative for a time and under conditions suitable for cell fixation to occur.

35

Fixing a cell ensures that the contents of the cell are less likely to be degraded and/or maintain their native conformation thereby facilitating detection.

5 A suitable compound for fixing a cell will be apparent to the skilled artisan and includes, for example, a compound selected from the group consisting of formaldehyde, paraformaldehyde, alcohol, methanol, glutaraldehyde, Bouin's fixative and mixtures thereof.

10 In one example of the invention, a cell is fixed at substantially the same time as the cell is permeabilized or disrupted. In another example, the cell is fixed prior to or after the cell is permeabilized or disrupted. In a further example, the cell is fixed in the absence of permeabilization or disruption.

15 Following permeabilization and/or fixation the level of a membrane transport protein is determined using a method known in the art and/or described *supra*.

20 Following determining the level of a membrane transport protein in a cell that comprises a membrane that has been permeabilized or disrupted, the level of the membrane protein at the surface of the protein relative to the level of membrane protein in a cell is determined. Accordingly, such a process enables a quantitative measurement of the level of a membrane transport protein that has translocated to the plasma membrane of a cell.

25 By determining the level of a membrane transport protein at the plasma membrane of a cell relative to or as a function of the level of the membrane transport protein in the cell, the process of the invention effectively standardizes or normalizes the detected levels of protein. The assay normalizes the level of translocated membrane transport protein based on the level of membrane transport protein in the assay. Such normalization facilitates comparison of results attained in separate/distinct assays.

30 Should the assay be performed using a plurality of cells, the assay may additionally be normalized, for example, for cell number. Such normalization accounts for variation in the number of cells in an assay (a variable that may affect the level of membrane protein detected in the assay).

35

Methods for determining cell number are known in the art, and include, for example, manually counting the number of cells used in an assay, or, alternatively, counting a fraction of the number of cells used in an assay. For example, when using a microtitre plate, the number of cells in a fraction of the total area of the plate (eg. 10% or 25% or 50%) of each well of the plate is counted, and this result used to estimate the number of cells in each well of the plate.

Alternatively, or in addition, a sample is normalized for cell number by detecting a protein that is expressed by the cells used in the assay. A protein useful in such an assay is one that is not affected by any conditions, eg., compounds, to which the cells are exposed. For example, should the cells be exposed to various concentrations of a compound, a protein that is affected by the compound (i.e., the expression levels of the protein) is not useful for normalization. Various proteins useful for normalization are known in the art and include, for example, β -tubulin, actin, glyceraldehyde 3-phosphate dehydrogenase (GAPDH), β 2 microglobulin, hydroxy-methylbilane synthase, hypoxanthine phosphoribosyl-transferase 1 (HPRT), ribosomal protein L13c, succinate dehydrogenase complex subunit A and TATA box binding protein (TBP).

Methods for determining the level of a protein are described *supra* and are to be taken to apply *mutatis mutandis* to the detection of a control protein for normalization. For example, the level of a control protein for normalization is determined using an antibody based assay.

In one example of the invention, the number of cells in a sample is determined by a method comprising contacting the cells with an antibody or ligand capable of binding to a component of the cell for a time and under conditions to occur and determining the level of antibody or ligand bound to the cells, wherein the level of antibody or ligand bound to the cells is indicative of cell number.

Antibodies capable of binding to such control proteins are known in the art. For example, an anti- β -tubulin monoclonal antibody is available from Sigma-Aldrich (Sydney, Australia), as is an anti-actin polyclonal antibody or an anti- β 2 microglobulin monoclonal antibody.

As the control proteins for normalization described *supra* are intracellular, such normalization is, for example, performed following disruption or permeabilization of the plasma membrane.

- 5 Alternatively, or in addition, the sample is normalized for cell number using a compound capable of passing across a cell membrane. For example, a DNA binding molecule, such as, for example Hoechst 33342, is capable of staining DNA in a cell with an intact plasma membrane. Clearly such a nucleic acid stain is also useful for normalization of a cell with a disrupted or permeabilized membrane. Alternative
10 nucleic acid stains include, for example, propidium-iodide, 4' 6-diamidino-2-phenylindole (DAPI), Mithramycin, 7-Aminoactinomycin D or To-Pro-3.

The present inventors have shown that wheat germ agglutinin (WGA) is also useful for normalization for cell number. WGA is capable of binding N-acetylglucosamine or
15 chitobiose. Both of these sugar structures are common to plasma membranes of many cells. Accordingly, WGA is useful for determining cell number or normalizing for cell number using either an undisrupted/unpermeabilized cell or a disrupted/permeabilized cell.

- 20 As will be apparent to the skilled artisan, the method need not determine or estimate the number of cells in a sample. Rather the method, for example, comprises determining the level of a ligand, antibody or compound used for detecting/estimating/normalizing for cell number in a sample and comparing this level to the level detected in another sample.

25

Accordingly, a method for normalizing for cell number comprises:

- (i) contacting a sample comprising a plurality of cells of the invention with a ligand or antibody capable of binding to a cell or a component thereof for a time and under conditions sufficient for a complex to form between the cell or component
30 thereof and the antibody or ligand and determining the level of the complex; and
(ii) contacting another sample comprising a plurality of cells of the invention with a ligand or antibody capable of binding to a cell or a component thereof for a time and under conditions sufficient for a complex to form between the cell or component thereof and the antibody or ligand and determining the level of the complex, wherein a
35 level of the complex that is similar or comparable in (i) and (ii) indicates that there is a similar or comparable number of cells in the samples.

For example, the level of the complex that is similar or comparable in (i) and (ii) does not vary significantly.

- 5 As will be apparent to the skilled artisan the level of the complex detected may also be used to normalize the level of translocated membrane transport protein detected. For example, the level of the translocated membrane transport protein detected is expressed as a function of the level of the complex detected thereby normalizing for approximate cell number.

10

Induction of translocation

- In an example of the invention, the process additionally comprises inducing translocation of the membrane transport protein. For example, the membrane transport protein is induced to translocate using a method comprising contacting a cell with an amount of peptide, polypeptide or protein sufficient to induce translocation of the membrane transport protein for a time and under conditions sufficient for translocation to occur thereby inducing translocation of the membrane transport protein.

- For example, contacting a cell with lactose or sucrose induces translocation of a lactose permease to a plasma membrane. Contacting a cell with a sufficient amount of isoproterenol induces translocation of the SCN5A sodium channel to the plasma membrane. Furthermore, contacting a cell with a secretagogue (e.g., KCl, ionomycin or a phorbol ester) induces translocation of a N-type Ca^{2+} channel to the plasma membrane of a cell.

25

Furthermore, the present inventors have shown that contacting a cell expressing a GLUT protein (e.g. a GLUT4 protein) with insulin induces increased translocation of the GLUT protein to the plasma membrane.

- 30 The present inventors have additionally demonstrated that by contacting a cell expressing a GLUT protein with an amount of insulin and sucrose to induce translocation enhanced levels of the GLUT protein are translocated to the plasma membrane. For example, levels of the GLUT protein translocated to the plasma membrane of a cell contacted with both sucrose and insulin are enhanced compared to the levels induced in a cell contacted with insulin alone.
- 35

Accordingly, the invention provides for induction of translocation of a GLUT protein or a mutant thereof by contacting a cell expressing said GLUT protein or mutant with an amount of insulin sufficient to induce translocation for a time and under conditions sufficient for translocation to occur.

5

In an example, the cell are additionally contacted with an amount of sucrose sufficient to induce translocation for a time and under conditions sufficient for translocation to occur.

- 10 In an example of the invention, a cell is contacted with sucrose and/or insulin in the presence of serum.

- In one form of the invention, the cells are contacted with insulin and then contacted with sucrose. For example, the cells are contacted with between about 100nM insulin
15 and about 700nM insulin, or between about 200nM insulin and about 600nM insulin, or about 200nM insulin, or about 400nM insulin or about 600nM insulin.

- Cells with an enhanced level of the membrane transport protein translocated to the plasma membrane are useful for, for example, screening for modulators of translocation
20 of the membrane transport protein. Clearly, such an assay is more sensitive than an assay that does not enhance the level of membrane transport protein at the cell surface. This is because the level of the plasma membrane transport protein at the cell surface is enhanced, thereby facilitating detection.

- 25 Furthermore, such an assay is useful for selecting for a potent inhibitor of translocation of a membrane transport protein.

- Furthermore, the present inventors have clearly demonstrated that the process of the invention is useful for screening for modulators of the level of translocation of a plasma
30 membrane protein. In particular, the present inventors have demonstrated that contacting a cell with insulin or contacting a cell with insulin and then sucrose are useful for enhancing the level of a GLUT4 protein translocated to the plasma membrane of a cell.

- 35 Alternative methods for the induction of translocation of GLUT4 to the plasma membrane include, for example, contacting a cell with a sufficient amount of

margatoxin or another voltage-gated K⁺ channel, Kv1.3 antagonist for a time and under conditions sufficient to suppress expression or activity of voltage-gated K⁺ channel, Kv1.3. Such suppression of activity (using margatoxin) or expression (using a mouse knock-out) has been shown to increase the level of GLUT4 translocated to the plasma membrane of a cell (Xu et al, *Proc Natl Acad Sci U S A.* 101:3112-3117, 2004.)

Suppression of translocation

The present inventors have additionally suppressed the level of a membrane transport protein translocated to the plasma membrane of a cell. Such a method is useful for, for example, modeling a disease/disorder or condition that is associated with a reduced or suppressed level of translocation of a plasma membrane protein. This model is then useful for determining a modulator or putative therapeutic of such a disease/disorder or condition.

For example, the present inventors have shown that by incubating cells expressing GLUT4 in the absence of insulin for a time and under conditions sufficient to induce resistance to insulin induced GLUT4 translocation the level of GLUT4 translocated to the plasma membrane of the cell in the presence of insulin is suppressed. For example, a cell is incubated in the presence of insulin for at least about 16 hours to at least about 72 hours prior to induction of translocation or testing of a compound/agent. For example, a cell is incubated in the presence of insulin for at least about 24 hours to at least about 48 hours prior to induction of translocation or testing of a compound/agent. For example, a cell is incubated in the presence of insulin for about 24 hours prior to induction of translocation or testing of a compound/agent. For example, a cell is incubated in the presence of insulin for about 48 hours prior to induction of translocation or testing of a compound/agent.

Conditions sufficient to induce resistance to insulin include, for example, the absence of insulin. Accordingly, an example of the invention provides for contacting a cell with insulin in the absence of serum for a time and under conditions to induce resistance to GLUT4 translocation. A cell that is resistant to insulin induced GLUT4 translocation is useful as a model for determining or identifying or isolating a modulator of insulin resistance, such as, for example, non-insulin dependent diabetes mellitus (NIDDM, type II diabetes).

Other methods for inducing resistance to translocation of a membrane transport protein will be apparent to those skilled in the art. For example, resistance to insulin induced translocation of a GLUT protein other than GLUT4 or a mutant thereof is induced using a method essentially as described *supra*.

5

Parallel cellular samples

One form of the present invention provides for performing the present invention in parallel cellular samples. Accordingly, the present invention provides a process for determining the level of a membrane transport protein translocated to the plasma
10 membrane of a cell, said process comprising:

- (a) determining the level of the membrane transport protein at the plasma membrane of a cell using a method comprising:
 - (i) contacting a cell with a ligand that binds to the extracellular domain of the membrane transport protein for a time and under conditions
15 sufficient for the ligand to bind the labeled membrane transport protein; and
 - (ii) determining the level of ligand bound to the membrane transport protein;
- (b) determining the level of membrane transport protein in another cell using a
20 method comprising:
 - (i) permeabilizing or disrupting the other cell;
 - (ii) contacting the membrane transport protein with the ligand for a time and under conditions sufficient for the ligand to bind the membrane transport protein;
 - (iii) determining the level of ligand bound to the membrane transport
25 protein; and
- (c) comparing the level of ligand detected at (a) (ii) and (b) (iii) to determine the level of the membrane transport protein at the plasma membrane relative to the total level of membrane transport protein.

30

As described *supra*, an example of the invention utilizes a labeled membrane transport protein to facilitate detection of the protein. Accordingly, the present invention provides a process for determining the level of a labeled membrane transport protein translocated to the plasma membrane of a cell, said process comprising:

- 35 (a) determining the level of the labeled membrane transport protein at the plasma membrane of a cell using a method comprising:

- (i) contacting a cell with a ligand that binds to the label for a time and under conditions sufficient for the ligand to bind the labeled membrane transport protein; and
 - (ii) determining the level of ligand bound to the labeled membrane transport protein;
- 5 (b) determining the level of labeled membrane transport protein in another cell using a method comprising:
 - (i) permeabilizing or disrupting the other cell;
 - (ii) contacting the labeled membrane transport protein with a ligand that
 - 10 binds to the label for a time and under conditions sufficient for the ligand to bind the labeled membrane transport protein;
 - (iii) determining the level of ligand bound to the labeled membrane transport protein; and
- 15 (c) comparing the level of ligand detected at (a) (ii) and (b) (iii) to determine the level of the labeled membrane transport protein at the plasma membrane relative to the total level of labeled membrane transport protein.

As used herein, the term "parallel cellular sample" shall be taken to mean that the cells used in the performance are grown under essentially or substantially the same

20 conditions. Accordingly, cells are grown in, for example, the same or similar growth medium and/or grown at approximately the same temperature and/or grown in the same concentration of CO₂. Preferably, the cells are also isogenic.

As used herein, the term "isogenic" shall be taken to refer to cells that are derived from

25 a clonal cell line. Accordingly, such cells are substantially identical at the genetic level. Preferably, each of the cells is from the same cell line.

For example, a cell that expresses a recombinant membrane transport protein preferably comprises an expression construct (encoding the recombinant membrane transport

30 protein) that has stably integrated into the genome of the cell. Such stable integration means that cells derived from the original cell also comprise the expression construct and express the encoded protein. Furthermore, stable integration of the expression construct facilitates a standard or relatively unvarying level of expression of the membrane transport protein in cells derived from the original cell.

By culturing cells in parallel comparisons are made more reproducible. This is because variables controlled or influenced by the environment in which a cell is grown or cultured, such as, for example, gene expression levels are essentially controlled. Accordingly, a direct comparison between the level of a membrane transport protein at the cell surface of one cell compared to the level of a membrane transport protein in another (isogenic) cell cultured under essentially the same conditions facilitates determining the level of the membrane transport protein translocated to the plasma membrane as a function of the level of the membrane transport protein in the cell.

10 Methods for determining the level of a ligand bound to a membrane transport protein and/or the level of a membrane transport protein are described *supra* and are to be taken to apply *mutatis mutandis* to the method for determining the level of a membrane transport protein translocated to the plasma membrane of a cell using a plurality of cells.

15 In one example, the process of the invention is performed in a plurality of cells. In accordance with this example, the inventive assay additionally comprises normalizing the determined level of ligand bound to the membrane transport protein with regard to the number of cells in which the level of the ligand bound to the membrane transport protein is determined. Methods for normalizing the determined level of ligand bound to the membrane transport protein are described *supra*.

20 Such normalization facilitates not only inter assay comparisons but also for determining the level of translocation of a membrane transport protein using cells cultured in, for example, parallel.

In an exemplified form of the invention, the inventors contacted a sample comprising cells with a labeled wheat germ agglutinin (WGA) for a time and under conditions sufficient for the WGA to bind to its ligand in the plasma membrane of a cell, and determining the level of WGA in the sample. For example, the sample is washed to remove any unbound WGA prior to detection. The level of WGA detected in the sample facilitates normalization of the level of the level of membrane transport protein detected relative to cell number. Clearly this facilitates determining the level of translocation of a membrane transport protein in addition to facilitating comparison between different samples.

Using the method of the present invention, the present inventors have produced a method for determining the level of a labeled GLUT4 protein or mutant thereof translocated to the plasma membrane of a cell. Accordingly, the present invention provides a process for determining the level of a labeled GLUT4 protein or labeled mutant GLUT4 protein translocated to the plasma membrane of a cell, said process comprising:

- (a) determining the level of the labeled GLUT4 protein or labeled mutant GLUT4 protein at the plasma membrane of a cell expressing the labeled GLUT4 protein or labeled mutant GLUT4 protein using a method comprising:
 - (i) contacting the cell with a ligand that binds to the label for a time and under conditions sufficient for the ligand to bind to the labeled GLUT4 protein or labeled mutant GLUT4 protein; and
 - (ii) detecting the level of ligand bound to the labeled GLUT4 protein or labeled mutant GLUT4 protein;
- (b) determining the level of membrane transport protein in another cell expressing the labeled GLUT4 protein or labeled mutant GLUT4 protein using a method comprising:
 - (i) permeabilizing or disrupting the other cell;
 - (ii) contacting the labeled GLUT4 protein or labeled mutant GLUT4 protein with a ligand that binds to the label for a time and under conditions sufficient for the ligand to bind to the labeled GLUT4 protein or labeled mutant GLUT4 protein;
 - (iii) detecting the level of ligand bound to the labeled GLUT4 protein or labeled mutant GLUT4 protein; and
- (c) comparing the level of ligand detected at (a) (ii) and (b) (iii) to determine the level of the labeled GLUT4 protein or labeled mutant GLUT4 protein at the plasma membrane relative to the total level of labeled GLUT4 protein or labeled mutant GLUT4 protein.

Furthermore, the present inventors have adapted this method to determine the level of a labeled GLUT4 protein or mutant thereof translocated to the plasma membrane of a cell that is resistant to insulin induced GLUT4 translocation. Accordingly, the present invention additionally provides a process for determining the level of the level of a labeled GLUT4 protein or a labeled mutant GLUT4 protein translocated to the plasma membrane of a cell that is resistant to insulin induced GLUT4 translocation, said process comprising:

- (a) contacting a plurality of cells expressing a labeled GLUT4 protein or a labeled mutant GLUT4 protein with insulin for a time and under conditions sufficient to induce resistance to insulin induced GLUT4 translocation in the cell;
- (b) determining the level of the labeled GLUT4 protein or labeled mutant GLUT4 protein at the plasma membrane of a cell (a) using a method comprising:
- 5 (i) contacting the cell with a ligand that binds to the label for a time and under conditions sufficient for the ligand to bind to the labeled GLUT4 protein or labeled mutant GLUT4 protein; and
- (ii) detecting the level of ligand bound to the labeled GLUT4 protein or labeled mutant GLUT4 protein;
- 10 (c) determining the level of membrane transport protein in another cell (a) using a method comprising:
- (i) permeabilizing or disrupting the other cell;
- (ii) contacting the labeled GLUT4 protein or labeled mutant GLUT4 protein with a ligand that binds to the label for a time and under conditions sufficient for the ligand to bind to the labeled GLUT4 protein or labeled mutant GLUT4 protein;
- 15 (iii) detecting the level of ligand bound to the labeled GLUT4 protein or labeled mutant GLUT4 protein; and
- 20 (d) comparing the level of ligand detected at (b) (ii) and (c) (iii) to determine the level of the labeled GLUT4 protein or labeled mutant GLUT4 protein at the plasma membrane relative to the total level of labeled GLUT4 protein or labeled mutant GLUT4 protein.

- 25 Methods for inducing resistance to GLUT4 translocation are described *supra* and are to be taken to apply *mutatis mutandis* to the instant example of the method of the invention.

As will be apparent to the skilled artisan the use of a labeled membrane transport protein is a model for the translocation of a wild-type or unlabeled membrane transport protein. For example, the label does not affect the function and/or translocation of the labeled membrane transport protein.

30

Determining recycling of a membrane transport protein

- 35 As a membrane transport protein is also recycled or turned-over from the plasma membrane of a cell (i.e. the membrane transport protein is removed from the

membrane) the present invention additionally provides a method for determining the level or rate of recycling of a membrane transport protein in a cell. Accordingly, the present invention additionally provides A process for determining the level of recycling of a membrane transport in a cell comprising:

- 5 (a) determining the level of the membrane transport protein translocated to the plasma membrane of a cell using the process of the invention;
- (b) determining the level of the membrane transport protein translocated to the plasma membrane of another cell using the process of the invention, wherein the other cell is cultured for a longer period of time than the cell (a); and
- 10 (c) comparing the level of the membrane transport protein translocated to the plasma membrane at (a) and (b) to determine the level of recycling of the membrane transport protein in the cell.

In another example, the present invention provides a process for determining a change
15 in the level of recycling of a membrane transport in a cell comprising:

- (a) determining the level of the membrane transport protein translocated to the plasma membrane of a cell using the process of the invention;
- (b) determining the level of the membrane transport protein translocated to the plasma membrane of another cell using the process of the invention, wherein the
20 other cell is cultured for a longer period of time than the cell (a); and
- (c) comparing the level of the membrane transport protein translocated to the plasma membrane at (a) and (b),

wherein a change in the level of the membrane transport protein translocated to the plasma membrane indicates a change in the level of recycling of a membrane
25 transport protein.

As will be apparent to the skilled artisan an increase in the level of the membrane transport protein translocated to the plasma membrane at (b) compared to (a) is indicative of an enhanced level of recycling of the membrane transport protein. In
30 contrast, a reduction in the level of the membrane transport protein at (b) compared to (a) is indicative of an enhanced level of recycling of the membrane transport protein.

By determining the change in the level of the membrane transport protein at the plasma membrane at (a) and (b) and optionally expressing this as a function the rate of
35 recycling of the membrane transport protein is determined. Clearly the present invention extends to determining the level of recycling of the membrane transport

protein at a number of points in time and determining the rate of recycling of the membrane transport protein.

5 In one form of the invention, the cells are contacted with the ligand of the label throughout the process. The present inventors have shown that following binding of the ligand to the label, recycling of the membrane transport protein is not altered.

10 The methods described *supra* are also useful for determining the rate and/or level of internalization of a membrane transport protein. For example, a cell is incubated in the presence of an agent that induces translocation of the membrane transport protein to the plasma membrane and then the agent is removed. By determining the level of the membrane transport protein at the plasma membrane at a plurality of points of time following the removal of the agent the level and/or rate of internalization of the membrane transport protein is determined.

15 Accordingly, the present invention provides a method for determining the level of internalization of a membrane transport protein comprising:

- 20 (a) inducing translocation of a membrane transport protein by a method comprising contacting a plurality of cells with one or more peptides, polypeptides, proteins or compounds that induces translocation of the membrane transport protein for a time and under conditions for translocation to occur;
- (b) determining the level of the membrane transport protein translocated to the plasma membrane of a cell (a) using the process of the invention;
- 25 (c) determining the level of the membrane transport protein translocated to the plasma membrane of another cell (a) using the process of the invention, wherein the other cell is cultured for a longer period of time than the cell (b); and
- (d) comparing the level of the membrane transport protein translocated to the plasma membrane at (b) and (c),

30 wherein the level of the membrane transport protein translocated to the plasma membrane at (b) compared to (c) indicates the level of internalization of the membrane transport protein.

Clearly this method applies *mutatis mutandis* to a method for determining the rate of internalization of a membrane transport protein.

35

Mutations affecting translocation of a membrane transport protein

The process of the present invention is also useful for determining or identifying a mutation in a nucleic acid that encodes a membrane transport protein wherein the mutation affects the translocation of the membrane transport protein. Accordingly, the present invention provides a method for determining a mutation in a nucleic acid
5 encoding a mutant membrane transport protein, wherein said mutation modulates translocation of said membrane transport protein, said method comprising:

- (a) determining the level of the mutant membrane transport protein translocated to the plasma membrane of a cell using the process of the invention; and
- (b) determining the level of a wild-type form of the membrane transport protein
10 translocated to the plasma membrane of a cell using the process of the invention, wherein an enhanced or suppressed level of translocation of the membrane transport protein at (a) compared to (b) indicates that the nucleic acid comprises a mutation that modulates the level of level of translocation of the membrane transport protein to the plasma membrane.

15

As will be apparent to the skilled artisan, this method may also be adapted to determine the level of recycling or internalization essentially as described *supra*.

In one form of the invention both the mutant and wild-type form of the membrane
20 transport protein are expressed in the same cell. As will be apparent to the skilled artisan, labeling each of the membrane transport proteins with a different label facilitates detection of each protein.

In another form of the invention, the mutant and wild-type form of the membrane
25 transport protein are expressed in different cells. Accordingly, each membrane transport protein may be with the same label.

In one form of the invention, the process additionally comprises providing a cell
30 expressing a mutant membrane transport protein and/or a wild-type form of the membrane transport protein. Methods for providing a cell, e.g. production of an expression construct and/or transforming/transfecting the expression construct into a cell are known in the art and described, for example, *supra*.

A mutant or mutated form of a membrane transport protein is isolated from a subject
35 suffering from, for example, a disorder thought to be associated with aberrant translocation of a membrane transport protein.

Alternatively, or in addition, a mutant form of a membrane transport protein is produced using recombinant means. Means for producing a mutation in a nucleic acid are known in the art and include for example, site-directed mutagenesis or PCR mediated mutagenesis. Such methods are described, for example, in Ausubel *et al* (In: Current Protocols in Molecular Biology. Wiley Interscience, ISBN 047 150338, 1987), Sambrook *et al* (In: Molecular Cloning: Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratories, New York, Third Edition 2001) or Dieffenbach (ed) and Dveksler (ed) (In: PCR Primer: A Laboratory Manual, Cold Spring Harbour Laboratories, NY, 1995).

The present inventors have produced various mutations in a cDNA encoding GLUT4 by, for example, site-directed mutagenesis or replacing regions of GLUT4 with regions from GLUT3. Furthermore, the present inventors have shown that these mutations affect the level of translocation of the mutant membrane transport protein.

In an example of the invention, the process additionally comprises determining the level of an expression product (e.g., mRNA or protein) encoded by the mutant and/or nucleic acid. Determining the level of expression of each nucleic acid facilitates comparing said expression levels to determine a compound that modulates the level of translocation of a membrane transport protein rather than modulating the level of expression of a membrane transport protein. Methods for determining expression levels are known in the art and/or are described, for example, in Ausubel *et al* (In: Current Protocols in Molecular Biology. Wiley Interscience, ISBN 047 150338, 1987), Sambrook *et al* (In: Molecular Cloning: Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratories, New York, Third Edition 2001) or Scopes (In: Protein purification: principles and practice, Third Edition, Springer Verlag, 1994).

Modulatory agents

The present invention provides an assay that is easily amenable to a process for the identification of compounds that modulate the level of translocation of a membrane transport protein. For example, the present inventors have shown that the process of the invention may be performed in a 384 well format thereby facilitating high-throughput screening for a modulatory compound. Accordingly, the present invention additionally provides a process for determining an agent that modulates translocation of

a membrane transport protein to the plasma membrane of a cell, said process comprising:

- (a) determining the level of a membrane transport protein translocated to the plasma membrane of a cell in the absence of a candidate agent by performing the process of the invention; and
- (b) determining the level of a membrane transport protein translocated to the plasma membrane of a cell in the presence of the candidate agent by performing the process of the invention,

wherein a difference in the level of a membrane transport protein translocated to the plasma membrane of a cell at (b) compared to (a) indicates that the candidate agent modulates translocation of the membrane transport protein.

As will be apparent to the skilled artisan an agent that enhances the level of membrane transport protein at (b) compared to (a) enhances the level of translocation of the membrane transport protein. In contrast an agent that reduces the level of membrane transport protein at (b) compared to (a) reduces the level of translocation of the membrane transport protein

The agent may be derived from any source. For example, a test agent can be a pharmacologic agent already known in the art or can be an agent previously unknown to have any pharmacological activity. The agent can be naturally occurring or designed in the laboratory. The agent can be isolated from microorganisms, animals, or plants, or can be produced recombinantly, or synthesized by chemical methods known in the art. If desired, test compounds can be obtained using any of the numerous combinatorial library methods known in the art, including but not limited to, biological libraries, spatially addressable parallel solid phase or solution phase libraries, synthetic library methods requiring deconvolution, the "one-bead one-compound" library method, and synthetic library methods using affinity chromatography selection. The biological library approach is limited to polypeptide libraries, while the other four approaches are applicable to polypeptide, non-peptide oligomer, or small molecule libraries of compounds. See Lam, *Anticancer Drug Des.* 12, 145: 1997.

Methods for the synthesis of molecular libraries are known in the art (see, for example, DeWitt *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 90: 6909, 1993; Erb *et al.* *Proc. Natl. Acad. Sci. U.S.A.* 91: 11422, 1994; Zuckermann *et al.*, *J. Med. Chem.* 37: 2678, 1994; Cho *et al.*, *Science* 261: 1303, 1993; Carell *et al.*, *Angew. Chem. Int. Ed. Engl.* 33: 2059, 1994;

Carell *et al.*, *Angew. Chem. Int. Ed. Engl.* 33: 2061; Gallop *et al.*, *J. Med. Chem.* 37: 1233, 1994). Libraries of compounds are, for example, presented in solution (see, e.g., Houghten, *Bio Techniques* 13: 412-421, 1992), or on beads (Lam, *Nature* 354: 82-84, 1991), chips (Fodor, *Nature* 364: 555-556, 1993), bacteria or spores (Ladner, U.S. Pat. No. 5,223,409), plasmids (Cull *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 89: 1865-1869, 1992), or: phage (Scott & Smith, *Science* 249: 386-390, 1990; Devlin, *Science* 249: 404-406, 1990); Cwirla *et al.*, *Proc. Natl. Acad. Sci.* 97: 6378-6382, 1990; Felici, J. *Mol. Biol.* 222: 301-310, 1991; and Ladner, U.S. Pat. No. 5,223,409).

10 Alternatively, an agent is isolated from a natural compound library. Such a natural compound library is commercially available from, for example, InterBioscreen, Moscow, Russia.

The present inventors have shown that the fungal metabolite wortmannin is capable of
15 suppressing GLUT4 translocation to the plasma membrane of a cell.

In one form of the invention a candidate agent is, for example an antibody or fragment thereof. Such an antibody is preferably capable of binding to and inhibiting the activity of a gene that is associated with or controls translocation of a membrane transport
20 protein to the plasma membrane of a cell.

For example, the membrane transport protein is GLUT4 and the antibody binds to voltage-gated K⁺ channel, Kv1.3 thereby inhibiting the activity of the channel. Inhibition of the activity of this ion channel has been previously shown to enhance
25 GLUT4 translocation to the plasma membrane.

In another form of the invention, the agent is an antisense nucleic acid, and RNAi molecule, a shRNA molecule or a ribozyme.

30 The term "antisense nucleic acid" shall be taken to mean DNA or RNA molecule that is complementary to at least a portion of a specific mRNA molecule (Weintraub, *Scientific American* 262:40, 1990) and capable of interfering with a post-transcriptional event such as mRNA translation. The use of antisense methods is known in the art (Marcus-Sakura, *Anal. Biochem.* 172: 289, 1988). Preferred antisense nucleic acid will
35 comprise a nucleotide sequence that is complementary to at least 15 contiguous nucleotides of a sequence encoding the amino acid of the protein of interest.

As used herein, the term "ribozyme" shall be taken to refer to a nucleic acid molecule having nuclease activity for a specific nucleic acid sequence. To achieve specificity, preferred ribozymes will comprise a nucleotide sequence that is complementary to at least about 12-15 contiguous nucleotides of a sequence encoding a protein that modulates the translocation of a membrane transport protein.

As used herein, the terms "small interfering RNA" ("siRNA"), short hairpin RNA ("shRNA"), and "RNAi" refer to homologous double stranded RNA (dsRNA) that specifically targets a gene product, thereby resulting in a null or hypomorphic phenotype. Specifically, the dsRNA comprises two short nucleotide sequences derived from the target RNA and having self-complementarity such that they can anneal, and interfere with expression of a target gene, presumably at the post-transcriptional level. RNAi molecules are described by Fire et al., Nature 391: 806-811, 1998, and reviewed by Sharp, Genes & Development, 13: 139-141, 1999). As will be known to those skilled in the art, short hairpin RNA ("shRNA") is similar to siRNA, however comprises a single strand of nucleic acid wherein the complementary sequences are separated an intervening hairpin loop such that, following introduction to a cell, it is processed by cleavage of the hairpin loop into siRNA. Accordingly, each and every embodiment described herein is equally applicable to siRNA and shRNA.

Preferred siRNA or shRNA molecules comprise a nucleotide sequence that is identical to about 19-21 contiguous nucleotides of the target mRNA. Preferably, the target sequence commences with the dinucleotide AA, comprises a GC-content of about 30-70% (preferably, 30-60%, more preferably 40-60% and more preferably about 45%-55%), and does not have a high percentage identity to any nucleotide sequence other than the target sequence in the genome of the animal in which it is to be introduced, e.g., as determined by standard BLAST search.

Methods for determining the level of translocation of a membrane transport protein are described *supra* and are taken to apply *mutatis mutandis* to the present method of the invention.

In one example, the method of the invention additionally comprises determining whether or not the agent is toxic. In accordance with this embodiment, the cells are screened to determine viability. Methods for determining viability include, for

example, contacting a cell with a labeled agent that is incorporated or taken up by the cell for a time and under conditions sufficient for the cell to take up or incorporate the agent and detecting the label. Alternatively, the method comprises contacting a cell with a compound that is metabolized by the cell for a time and under conditions
5 sufficient for the cell to metabolize the compound and detecting the metabolite.

For example, a cell viability assay comprises determining the level of ^3H thymidine by a cell. Alternatively, trypan blue staining is useful for determining cell viability. Alternatively, or in addition, colorimetric assays such as for example, the ProCheckTM
10 assay is available from Serologicals. A variety of other cell viability assays are known in the art and described for example, in *Animal Cell Culture: Practical Approach*, Third Edition (John R.W. Masters, ed., 2000), ISBN 0199637970.

For example, cell viability is measured using a methylthiazol tetrazolium (MTT)
15 reduction assay (Mossman, *J. Immunol. Meth.*, 65: 55, 1983). MTT is reduced by mitochondrial dehydrogenases in living cells; this reaction produces formazan crystals which are quantified by photometry after extraction. For example, using this method, an IC₅₀ (concentration that reduces cell viability by 50 %) is calculated.

20 Neutral red staining is also useful for determining cell viability. Neutral red is accumulated in the lysosomes in living cells that become colored by the dye. The dye is extracted and quantified using densitometry.

Alternatively, or in addition, cell viability is determined by determining the level of
25 lactate dehydrogenase activity (Legrand *et al.*, *J. Biotechnol.* 25:231-43, 1992). Lactate Dehydrogenase is a cytosolic enzyme that is released upon cell lysis. For example, an IC₅₀ (concentration that reduces cell viability by 50 %) can be calculated. This assay evidences chemicals inducing alterations in cell integrity (lysis). Kits for determining lactate dehydrogenase levels are commercially available from, for example, Promega or
30 Vinci-Biochem, Vinci, Italy.

In one example, the present invention provides a process for determining an agent that modulates translocation of a membrane transport protein to the plasma membrane of a cell, said process comprising:

- (a) determining the level of a membrane transport protein translocated to the plasma membrane of a cell in the absence of a candidate agent by performing the process of the invention;
- (b) determining the level of a membrane transport protein translocated to the plasma membrane of a cell in the presence of the candidate agent by performing the process of the invention, wherein a difference in the level of a membrane transport protein translocated to the plasma membrane of a cell at (a) compared to (b) indicates that the candidate agent modulates translocation of the membrane transport protein.
- (c) optionally, determining the structure of the candidate agent; and
- (d) providing the candidate agent or the name or structure of the candidate agent.

Naturally, for agents that are known albeit not previously tested for their function using a screen provided by the present invention, determination of the structure of the compound is implicit in step (i) *supra*. This is because the skilled artisan will be aware of the name and/or structure of the compound at the time of performing the screen.

As used herein, the term "providing the agent" shall be taken to include any chemical or recombinant synthetic means for producing said agent or alternatively, the provision of an agent that has been previously synthesized by any person or means.

For example, a peptidyl compound is synthesized using is produced synthetically. Synthetic peptides are prepared using known techniques of solid phase, liquid phase, or peptide condensation, or any combination thereof, and can include natural and/or unnatural amino acids. Amino acids used for peptide synthesis may be standard Boc (N α -amino protected N α -t-butyloxycarbonyl) amino acid resin with the deprotecting, neutralization, coupling and wash protocols of the original solid phase procedure of Merrifield, *J. Am. Chem. Soc.*, 85:2149-2154, 1963, or the base-labile N α -amino protected 9-fluorenylmethoxycarbonyl (Fmoc) amino acids described by Carpino and Han, *J. Org. Chem.*, 37:3403-3409, 1972. Both Fmoc and Boc N α -amino protected amino acids can be obtained from various commercial sources, such as, for example, Fluka, Bachem, Advanced Chemtech, Sigma, Cambridge Research Biochemical, Bachem, or Peninsula Labs.

Synthetic peptides are alternatively produced using techniques known in the art and described, for example, in Stewart and Young (*In: Solid Phase Synthesis*, Second

Edition, Pierce Chemical Co., Rockford, Ill. (1984) and/or Fields and Noble (*Int. J. Pept. Protein Res.*, 35:161-214, 1990), or using automated synthesizers. Accordingly, peptides of the invention may comprise D-amino acids, a combination of D- and L-amino acids, and various unnatural amino acids (e.g., β -methyl amino acids, α -methyl amino acids, and $N\alpha$ -methyl amino acids, etc) to convey special properties. Synthetic amino acids include ornithine for lysine, fluorophenylalanine for phenylalanine, and norleucine for leucine or isoleucine.

In another embodiment, a peptidyl agent is produced using recombinant means. For example, an oligonucleotide or other nucleic acid (eg., a nucleic acid encoding a dominant negative inhibitor of the protein of interest) is placed in operable connection with a promoter. Methods for producing such expression constructs, introducing an expression construct into a cell and expressing and/or purifying the expressed peptide, polypeptide or protein are known in the art and described *supra*.

Alternatively, the peptide, polypeptide or protein is expressed using a cell free system, such as, for example, the TNT system available from Promega. Such an *in vitro* translation system is useful for screening a peptide library by, for example, ribosome display, covalent display or mRNA display.

Methods for producing antibodies, preferably a monoclonal antibody, or a fragment or recombinant fragment thereof are described *supra*.

In a preferred embodiment, the compound or modulator or the name or structure of the compound or modulator is provided with an indication as to its use e.g., as determined by a screen described herein.

In another example, the invention provides a process for determining an agent that modulates translocation of a membrane transport protein to the plasma membrane of a cell, said process comprising:

- (a) determining the level of a membrane transport protein translocated to the plasma membrane of a cell in the absence of a candidate agent by performing the process of the invention;
- (b) determining the level of a membrane transport protein translocated to the plasma membrane of a cell in the presence of the candidate agent by performing the process of any one of the invention, wherein a difference in the level of a

membrane transport protein translocated to the plasma membrane of a cell at (a) compared to (b) indicates that the candidate agent modulates translocation of the membrane transport protein.

- (c) optionally, determining the structure of the candidate agent;
- 5 (d) optionally, providing the name or structure of the candidate agent; and
- (d) providing, the candidate agent.

In one example, the candidate agent is provided with an indication as to its use, for example, as determined using a method described herein.

10

The present inventors have additionally produced a method for modeling insulin resistance. For example, the present inventors have produced a model in which a cell is resistant to insulin induced GLUT4 translocation. Accordingly, the present invention additionally provides a process for determining a candidate compound for the treatment

15 of insulin resistance comprising:

- (a) contacting a plurality of cells expressing a labeled GLUT4 protein or a labeled mutant GLUT4 protein with insulin for a time and under conditions sufficient to induce resistance to insulin induced GLUT4 translocation in the cell;
- (b) determining the level of the labeled GLUT4 protein or a labeled mutant GLUT4
- 20 protein translocated to the plasma membrane of a cell (a) in the absence of a candidate agent by performing the process of the invention; and
- (c) determining the level of the labeled GLUT4 protein or a labeled mutant GLUT4 protein translocated to the plasma membrane of another cell (a) in the presence of the candidate agent by performing the process of the invention,
- 25 wherein a candidate agent that enhances the level of translocation of the labeled GLUT4 protein or a labeled mutant GLUT4 protein is a candidate agent for the treatment of insulin resistance.

Conditions associated with insulin resistance include, for example, Syndrome X, type II

30 diabetes (non-insulin dependent diabetes mellitus (NIDDM), hypertension, cardiovascular disease or obesity. Accordingly, an agent identified or determined using the method of the present invention is, for example, useful for the treatment of such a condition.

35 In one example, the agent is provided with an indication as to its use, for example, as determined using a method described herein.

The present invention additionally provides a process for determining a candidate compound for the treatment of insulin resistance comprising:

- 5 (a) contacting a plurality of cells expressing a labeled GLUT4 protein or a labeled mutant GLUT4 protein with insulin for a time and under conditions sufficient to induce resistance to insulin induced GLUT4 translocation in the cell;
- (b) determining the level of the labeled GLUT4 protein or a labeled mutant GLUT4 protein translocated to the plasma membrane of a cell (a) in the absence of a candidate agent by performing the process of the invention;
- 10 (c) determining the level of the labeled GLUT4 protein or a labeled mutant GLUT4 protein translocated to the plasma membrane of another cell (a) in the presence of the candidate agent by performing the process of the invention, wherein a compound that enhances the level of translocation of the labeled GLUT4 protein or a labeled mutant GLUT4 protein is a candidate compound for the treatment
- 15 of diabetes;
- (d) optionally, determining the structure of the candidate agent; and
- (e) providing the candidate agent or the name or structure of the candidate agent.

In one example, the agent is provided with an indication as to its use, for example, as
20 determined using a method described herein.

Furthermore, the present invention provides a process for determining a candidate compound for the treatment of insulin resistance comprising:

- 25 (a) contacting a plurality of cells expressing a labeled GLUT4 protein or a labeled mutant GLUT4 protein with insulin for a time and under conditions sufficient to induce resistance to insulin induced GLUT4 translocation in the cell;
- (b) determining the level of the labeled GLUT4 protein or a labeled mutant GLUT4 protein translocated to the plasma membrane of a cell (a) in the absence of a candidate agent by performing the process of the invention;
- 30 (c) determining the level of the labeled GLUT4 protein or a labeled mutant GLUT4 protein translocated to the plasma membrane of another cell (a) in the presence of the candidate agent by performing the process of the invention, wherein a compound that enhances the level of translocation of the labeled GLUT4 protein or a labeled mutant GLUT4 protein is a candidate compound for the treatment
- 35 of diabetes;
- (d) optionally, determining the structure of the candidate agent;

- (e) optionally, providing the name or structure of the candidate agent; and
- (e) providing the candidate agent.

Suitable agents are known in the art and/or described *supra*.

5

Furthermore, methods for determining the level of translocation of a labeled GLUT4 protein or a labeled mutant GLUT4 protein translocated to the plasma membrane of a cell are known in the art and/or described herein.

- 10 For example, the method of the invention is useful for determining an agent for the treatment of diabetes, e.g., NIDDM.

Accordingly, the present invention additionally provides a process for manufacturing a medicament for the treatment of insulin resistance comprising:

- 15 (a) determining a candidate compound for the treatment of insulin resistance using a process comprising:
- (i) contacting a plurality of cells expressing a labeled GLUT4 protein or a labeled mutant GLUT4 protein with insulin for a time and under conditions sufficient to induce resistance to insulin induced GLUT4
 - 20 translocation in the cell;
 - (ii) determining the level of the labeled GLUT4 protein or a labeled mutant GLUT4 protein translocated to the plasma membrane of a cell (a) in the absence of a candidate agent by performing the process of the invention;
 - (iii) determining the level of the labeled GLUT4 protein or a labeled mutant
 - 25 GLUT4 protein translocated to the plasma membrane of another cell (a) in the presence of the candidate agent by performing the process of the invention, wherein a compound that enhances the level of translocation of the labeled GLUT4 protein or a labeled mutant GLUT4 protein is a candidate compound for the treatment of diabetes;
- 30 (b) optionally, isolating the candidate agent;
- (c) optionally, providing the name or structure of the candidate agent;
 - (d) optionally, providing the candidate agent; and
 - (e) using the candidate agent in the manufacture of a medicament for the treatment of insulin resistance.

35

Suitable agents and methods for determining their affect on GLUT4 translocation are described *supra*. Additionally, methods for inducing insulin resistance in a cell are described *supra*. For example, the cell is treated with insulin in the absence of serum for a time and under conditions sufficient to induce resistance to insulin induced
5 GLUT4 translocation in the cell.

For example, the agent is formulated into a pharmaceutical formulation. Formulation of a pharmaceutical compound will vary according to the route of administration selected (e.g., solution, emulsion, capsule). An appropriate composition comprising the
10 identified modulator to be administered can be prepared in a physiologically acceptable vehicle or carrier. For solutions or emulsions, suitable carriers include, for example, aqueous or alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles can include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's or fixed oils, for instance.
15 Intravenous vehicles can include various additives, preservatives, or fluid, nutrient or electrolyte replenishers and the like (See, generally, Remington's Pharmaceutical Sciences, 17th Edition, Mack Publishing Co., Pa., 1985). For inhalation, the agent can be solubilized and loaded into a suitable dispenser for administration (e.g., an atomizer, nebulizer or pressurized aerosol dispenser).

20 Furthermore, where the agent is a protein or peptide or antibody or fragment thereof, the agent can be administered via *in vivo* expression of the recombinant protein. *In vivo* expression can be accomplished via somatic cell expression according to suitable methods (see, e.g. U.S. Pat. No. 5,399,346). In this embodiment, nucleic acid encoding
25 the protein can be incorporated into a retroviral, adenoviral or other suitable vector (preferably, a replication deficient infectious vector) for delivery, or can be introduced into a transfected or transformed host cell capable of expressing the protein for delivery. In the latter embodiment, the cells can be implanted (alone or in a barrier device), injected or otherwise introduced in an amount effective to express the protein
30 in a therapeutically effective amount.

The pH and exact concentration of the various components the formulation suitable for administration to the animal are adjusted according to routine skills in the art.

35 Following determination of an agent using a method described herein, the agent is additionally tested *in vivo*. For example, a candidate agent for the treatment of a mouse

or rat model of NIDDM. For example, a mouse model is a mouse, such as for example a Cpe^{fat} mouse, a Lep^{ob} mouse, a Lepr^{ob} mouse or a tub mouse (all available from Jackson Laboratories). Alternative models of NIDDM include, for example, the tallyho mouse (Kim *et al.*, *Genomics* 74: 273-286, 2001) or the OLETF rat (Watanabe *et al.*,
5 *Genomics* 58: 233-239). Such models are useful for, for example, determining the toxicity of a compound and/or the efficacy of a compound (e.g., the level or amount of the compound required for treatment).

The present invention is further described with reference to the following non-limiting
10 examples

EXAMPLE 1

GENERATION AND EXPRESSION OF A LABELED GLUT4 PROTEIN

A HA-tagged GLUT4 protein was produced essentially as described in Quon *et al.*,
5 *Proc. Natl. Acad. Sci USA* 94: 5587-5591, 1994. Essentially, the cDNA encoding
GLUT4 was digested with *SauI* and a double stranded oligonucleotide was inserted by
ligation. The double stranded oligonucleotide was formed by hybridizing two
oligonucleotides one comprising the sequence
TGAGATCGATTATCCTTATGATGTTCTGATTATGG (SEQ ID NO: 63) and the
10 other TCA GCA TAA TCA GGA ACA TCA TAA GGA TAA TCG ATC (SEQ ID
NO: 64). The inserted nucleic acid encodes a HA tag between amino acids 67 and 68
in the first exofacial loop of GLUT4 (SEQ ID NO: 4). This gene construct was inserted
into the vector pBABE (Pear *et al. Proc. Natl Acad. Sci. U.S.A.* 90: 8392-8396 1993).
The polypeptide encoded by this protein is shown schematically in Figure 1A.

15 Additional gene constructs were generated comprising a nucleic acid encoding mutant
forms of GLUT4 (these constructs encoded the TAIL mutant of GLUT4 (SEQ ID NO:
5), the L489,490A mutant of GLUT4 (SEQ ID NO: 7) and the F5A mutant of GLUT4
(SEQ NO: 9), each tagged with a HA tag), comprising a HA tag in the first
20 extracellular domain of the protein, essentially as described in Piper *et al, The Journal
of Cell Biology*, 121(6):1221-1232, 1993, Marsh *et al, JCB*, 130(5): 1081-1091, 1995,
Shewan *et al. Biochem. J.* 350: 99-107, 2000 and Shewan *et a, Mol. Biol. Of Cell*, 14:
973-986, 2003. The proteins encoded by these nucleic acids are schematically
represented in Figure 1B.

25 Retroviral stocks of each of the constructs were produced using the method described
in Pear *et al. Proc. Natl Acad. Sci. U.S.A.* 90: 8392-8396 1993. To generate 3T3-L1
adipocytes stably expressing the each construct 3T3-L1 fibroblasts (plated at a density
of 5×10^5 /100mm plate 16 h beforehand) were infected with the relevant virus for 3-5h
30 in the presence of 4µg/ml Polybrene (Sigma). After a 48h recovery period, infected
cells were then selected in DMEM containing 10% FCS and supplemented with 2µg/ml
puromycin (Sigma).

3T3-L1 fibroblasts up to passage 20 were cultured in high glucose DMEM
35 supplemented with 10% heat-inactivated new born calf serum (NCS) at 37°C in 5%
CO₂. For differentiation into adipocytes, fibroblasts were cultured in DMEM/NCS for

up to one or two days post-confluence, after which the cells were cultured for three days in DMEM containing 10% heat-inactivated fetal bovine serum (FBS), 350 nM insulin, 0.5 mM 3-isobutyl-1-methylxanthine (IBMX), 250 nM dexamethasone, 400 nM biotin and for three days in DMEM containing 10% FBS and 350 nM insulin. After
5 differentiation, adipocytes were maintained in DMEM supplemented with 10% FBS. Adipocytes were used for experiments 8 to 11 days after the onset of differentiation and the medium was renewed two or three days prior to each experiment. For culturing in gelatin-coated 96 well plates, cells were seeded at a 1:1 cell surface ratio and differentiation was initiated four days post-seeding.

10 To determine expression of the constructs transduced cells were studied using immunofluorescence. Cells were stained for either the HA tag (Covance, Berkeley, CA, USA) or anti-GLUT4 (Martin *et al.*, *J. Cell Biol.* 134: 625-635, 1994). As shown in Figure 1D approximately 90% of cells expressed the recombinant HA-GLUT4.

15 Steady state labeling of unstimulated cells revealed a predominant perinuclear GLUT4 localization in fibroblasts with low levels of GLUT4 in small peripheral vesicles. GLUT4 TAIL was more concentrated in peripheral vesicles compared to wild-type GLUT4 when expressed in fibroblasts (Fig. 1G).

20 Expression levels of the expression of the recombinant forms of GLUT4 was then determined using immunoblotting. Confluent 3T3-L1 fibroblasts and 3T3-L1 adipocytes at day 8 of differentiation were serum-starved for 2 h and lysed in PBS containing 1% Triton X-100, 1 mM EDTA, 1 mM PMSF, 10µg/ml aprotinin and
25 10µg/ml leupeptin. Equal amounts of protein were subjected to SDS- PAGE and transferred to PVDF membrane. Membranes were incubated with the indicated antibodies. HRP-conjugated secondary antibodies were visualized using ECL reagent (Pierce, Rockford, IL) and a 16 bit camera-based imager (VersaDoc 5000; Bio-Rad, Regents Park, Australia). For quantitation, a serial dilution of a control sample was run
30 on the same SDS-PAGE gel and Quantity One software (Bio-Rad, Regents Park, Australia) was used for analysis. An anti-HA immunoblot was used to determine the relative expression of GLUT4 TAIL as this GLUT4 molecule was not recognized by the anti-GLUT4 antibody.

There was a modest level of overexpression (Fig. 1E and 1F), making it unlikely that GLUT4 localization was disturbed due to saturation of the cellular trafficking machinery.

5

EXAMPLE 2

GENERATION OF AN ASSAY TO DETERMINE THE LOCALIZATION OF GLUT4

2.1 Methods

10 Retrovirally-transduced fibroblasts expressing HA-tagged GLUT 4 or a mutant thereof were differentiated into adipocytes essentially as described above. These adipocytes were then subcultured for 30 hours. Insulin was then added at different time points, after which the cells were fixed in 3% formaldehyde. After washing and quenching with 50 mM glycine, cells were incubated for 20 min with 5% normal swine serum
15 (NSS) in the absence or presence of 0.1% saponin to analyse the level of GLUT4 at the plasma membrane (PM) or the total cellular GLUT4 content, respectively. Cells were incubated for 60 min with a saturating concentration of either an antibody directed against the HA tag or a control non-relevant antibody (mouse IgG MOPC21) in PBS containing 2% NSS. After extensive washing, the cells were incubated for 20 min with
20 5% NSS in the presence or absence of 0.1% saponin to permeabilize all cells. Cells were incubated for 60 min with saturating concentrations of ALEXA488-conjugated goat-anti-mouse antibody (20 µg/ml) and ALEXA594-conjugated WGA (10 µg/ml) in PBS containing 2% NSS. After washing, fluorescence (emm 485/exc 520 and emm 544/exc 630) was measured using the bottom-reading mode in a fluorescence microtiter
25 plate reader (FLUOstar Galaxy, BMG Labtechnologies, Offenburg, Germany). The percentage of GLUT4 at the PM was calculated for each condition. ALEXA594-WGA fluorescence was used to correct for variation in cell density in each well.

2.2 Results

30 To determine the extent of insulin-induced GLUT4 translocation using the assay described *supra*, HA-GLUT4-expressing 3T3-L1 adipocytes grown in 96 well plates were incubated for 2 h in the absence of serum, whereafter 200 nM insulin was added at various time points and cell surface levels of HA-GLUT4 were analysed by indirect immunofluorescence labeling (Fig. 2B). Saturating levels of anti-HA and secondary
35 antibodies were used to ensure that substantially all HA-GLUT4 molecules were labeled. A non-relevant antibody was used at the same concentration to determine the

non-specific binding of the anti-HA antibody. Insulin stimulated the appearance of HA-GLUT4 at the PM with a half-time of about 2.5 min reaching a plateau by 12 min, which was maintained for at least 60 min. No specific anti-HA labeling was detected in non-infected cells (Fig. 2A). Expressing the amount of specific fluorescence at the PM as a percentage of the total specific fluorescence revealed that insulin increased the level of GLUT4 at the PM from a basal value of 4% up to 34% (Fig. 2C) and this effect was inhibited by wortmannin (Fig. 2D).

EXAMPLE 3

10 Insulin induced translocation of GLUT4 in 3T3-L1 fibroblasts and adipocytes

In fibroblasts, insulin induced the translocation of wild-type GLUT4 and each of the GLUT4 mutants to the PM (Fig. 3). The maximum level of surface GLUT4 was reached after 6 min of insulin stimulation, representing a 5-fold increase above that observed in non-stimulated cells, followed by a rapid reduction. The PM level of the GLUT4 F5A mutant was slightly higher than that of the other GLUT4 molecules in insulin-stimulated fibroblasts. In adipocytes we observed an ~8-fold increase in cell surface GLUT4 levels in response to insulin stimulation. Neither wild-type GLUT4 nor any of the GLUT4 mutants showed an overshoot as was observed in fibroblasts. The GLUT4 TAIL mutant showed translocation characteristics similar to those of GLUT4 WT, although cell surface levels in both the absence and presence of insulin were increased by approximately 5%, in accordance with previous studies (Shewan *et al.*, *Mol. Biol. Cell* 14: 973-986, 2003). The PM levels of both the L489,490A and F5A mutants were significantly higher than those of GLUT4 WT, both in the absence and presence of insulin.

EXAMPLE 4

GLUT4 internalization and recycling in 3T3-L1 adipocytes

30 4.1 Methods

For single cycle internalization experiments cells were stimulated for 20 min with 200 nM insulin after starvation and washed on ice with ice-cold DMEM containing 20 mM HEPES pH 7.4 and 0.2% BSA. Cells were incubated with 100 nM wortmannin or 200 nM insulin and either anti-HA (25 µg/ml) or non-relevant antibody (MOPC21) in DMEM/HEPES/BSA for 1 h on ice. Wortmannin was added to abolish insulin signalling. This drug has no direct effect on GLUT4 internalization in adipocytes

(Malide and Cushman *J. Cell Sci.* 110: 2795-2806) and has previously been used to study GLUT4 internalization (Al-Hasani *et al.*, *J. Biol. Chem.* 273: 17504-17510). Cells were washed extensively, then either 100 nM wortmannin or 200 nM insulin in DMEM/HEPES/BSA was added. The plate was then transferred to 37°C and at
5 different times, formaldehyde was added to the wells to a concentration of 3%. After 5 min the formaldehyde was washed away and residual amounts were quenched. The cells were incubated for 20 min with 5% NSS in the absence of saponin, labeled with ALEXA488-conjugated goat-anti-mouse antibody and ALEXA594-conjugated WGA, washed and analysed as described above.

10

For continuous antibody uptake experiments, cells were incubated for 20 min with or without insulin, whereafter anti-HA (50 μ g/ml) or non-relevant antibody was added. Cells that were used to determine the total amount of HA-GLUT4 were not incubated with antibody during this 37°C incubation. After incubation, the cells were fixed and
15 quenched as described above, and incubated for 20 min with 5% NSS and 0.1% saponin. Cells that were used to determine the total cellular amount of HA-GLUT4 were incubated for 60 min with anti-HA antibody or control antibody in PBS containing 2% NSS. All other cells were incubated with 2% NSS without antibody. Subsequently, the cells were incubated with ALEXA488-conjugated goat-anti-mouse
20 antibody and ALEXA594-conjugated WGA, washed and analysed. The amount of specific anti-HA uptake was expressed as a percentage of total cellular immuno-reactive HA-GLUT4.

4.2 Analysis of GLUT4 internalization in 3T3-L1 adipocytes

25 GLUT4 WT molecules that were labeled with anti-HA antibody on ice were rapidly cleared from the cell surface as indicated by the disappearance of GLUT4 at early time points after transfer of the cells from ice to 37°C (Fig. 4). After approximately 5 min the level of GLUT4 at the PM reached steady state in the presence but not in the absence of insulin, indicating recycling of GLUT4 back to the PM in insulin-stimulated
30 cells. Our data indicated that after 2 min at 37°C ~50% of both GLUT4 WT and GLUT4 TAIL had disappeared from the PM. Importantly, this internalization rate was unaffected by insulin, consistent with previous studies (Sato *et al.*, *J. Biol. Chem.* 268: 17820-17829, 1993). The internalization rates for the L489,490A and F5A mutants were decreased by 30 and 45%, respectively (Fig. 4).

35

4.3 Anti-HA antibody uptake by HA-GLUT4-expressing fibroblasts and adipocytes

To analyze the exchange of GLUT4 with the cell surface under steady state conditions, studies were performed in which live cells were incubated with anti-HA antibody at 37°C (Fig. 5). To ascertain that the anti-HA antibody did not affect the intracellular trafficking of HA-GLUT4, control experiments were performed in which insulin-induced translocation of anti-HA-bound HA-GLUT4 was studied. 3T3-L1 adipocytes expressing HA-GLUT4 WT were stimulated for 2 h with 200 nM insulin in the presence of anti-HA antibody, washed extensively, incubated for 2 h without insulin and anti-HA, and incubated for a further 20 min in the absence (Fig. 5C) or presence (Fig. 5D) of 200 nM insulin. The cells showed insulin-induced redistribution of anti-HA-bound HA-GLUT4 from intracellular compartments to the PM that was indistinguishable from translocation of HA-GLUT4 that had not been pre-labeled with antibody (Fig. 5A and 5B), indicating that the anti-HA antibody had no significant effect on GLUT4 trafficking.

For quantification of anti-HA antibody uptake, cells were preincubated for 20 min in the presence or absence of insulin after which anti-HA antibody or control antibody was added for various times (Fig. 5E). Antibody uptake was determined by labeling cells with fluorescent secondary antibody after fixation. Antibody uptake was expressed as a percentage of post-fixation anti-HA labeling.

20

Several observations were made from these studies. Firstly, there was a profound difference in recycling kinetics for HA-GLUT4 between fibroblasts and adipocytes in the absence of insulin. Whereas in fibroblasts a significant portion of the GLUT4 molecules recycled between intracellular compartments and the PM in the absence of insulin (~50% after 60 min), this was not the case in adipocytes with only ~10% of the entire GLUT4 pool labeled after 3 h. A similar percentage of GLUT4 was labeled after 6 h (not shown). Recycling of HA-GLUT4 in the presence of insulin was similar for fibroblasts and adipocytes. Secondly, the recycling rate of HA-GLUT4 TAIL in non-stimulated adipocytes was significantly higher than that observed for GLUT4 WT.

30

Thirdly, both of the internalization mutants showed a minor increase in basal anti-HA uptake and no difference in uptake during insulin stimulation compared with GLUT4 WT. Finally, it was noted that even with maximum insulin stimulation a small but significant pool of GLUT4 did not exchange with the cell surface under steady state conditions. The size of this pool was similar between fibroblasts and adipocytes and for

35

the different GLUT4 mutants suggesting that it represents a pool of GLUT4 that is segregated from the insulin responsive pool.

To study this non-recycling GLUT4 pool in adipocytes, 3T3-L1 adipocytes expressing
5 HA-GLUT4 WT were incubated at 37°C in the continuous presence of anti-HA
antibody. Cells were incubated with or without 200 nM insulin for 20 min, after which
anti-HA antibody was added in the continued presence or absence of insulin. Cells
were incubated further for up to 180 min, fixed, permeabilized, and incubated with
10 fluorescent secondary antibody. The level of anti-HA antibody taken up by the cells
was then expressed as a percentage of total post-fixation anti-HA labeling of
permeabilized cells. As shown in Fig 6A, only approximately 30% of the HA-GLUT4
detected in a cell is labeled in the insulin induced cells. This suggests that
approximately 30% of the HA-GLUT4 expressed in the cell did not translocate to the
membrane during the experiment.

15 The cells that were used to determine the 100% value of HA-GLUT4 that recycled to
the plasma membrane were incubated again with fixative after the post-fixation anti-
HA immunolabeling. As shown in Fig 6B fixation of the anti-HA antibody appeared
not to change the affinity of the secondary antibody and therefore did appear not cause
20 the 30% of difference in labeling.

Cells were again incubated with anti-HA after fixation without permeabilization. As
shown in Fig 6C the 30% of HA-GLUT4 that cannot be labeled with antibody during
the 37°C incubation is not present at the cell surface. Furthermore, cells were incubated
25 again with the anti-HA antibody after fixation and permeabilization. In this case, 100%
of GLUT4 was labeled, indicating that the 30% of HA-GLUT4 that cannot be labeled
during the continuous antibody uptake is not unable to bind antibody but remains
intracellular during the antibody uptake incubation.

30 To determine whether or not the antibody concentration used limited the level of HA-
GLUT4 detected in a cell, cells were incubated for 3 h in the presence of insulin with
various concentrations of anti-HA (in this regard, the standard concentration used was
50 mg/ml). As shown in Figure 6E antibody concentration during the antibody
incubation appeared not to be limiting with comparable levels of HA-GLUT4 being
35 detected with various concentrations of anti-HA antibody.

To determine whether or not the unlabeled HA-GLUT4 was still in the process of synthesis or part of the biosynthetic tract cells were incubated with 10 mg/ml cycloheximide for 2 h prior to the addition of antibody. As shown in Figure 6F 30% of GLUT4 could not be labeled, suggesting that the non-labeled GLUT4 pool is not part
5 of the biosynthetic tract.

To determine the effect of endosomal pH on the binding of anti-HA antibody to HA-GLUT4 was determined. Cells were incubated for 30 min at 37°C in hypertonic medium (0.45 M sucrose, pH 7.4), on ice with antibody in the same medium, and at
10 37°C in hypertonic buffer at pH 7.4 or pH 5.5 in the absence of antibody. Release of antibody from the plasma membrane at neutral or endosomal pH was determined by incubating fixed non-permeabilized cells with fluorescent secondary antibody. As shown in Figure 6G, endosomal pH did not induce the release of the anti-HA antibody from the HA-tag.

15 The effect of long-term insulin treatment on the amount of cell surface HA-GLUT4 levels was also determined. In this regard, cells were incubated for various times with 200 nM insulin and cell surface GLUT4 levels were determined as described *supra*. As shown in Figure 6H, insulin did not drastically down-regulate cell surface GLUT4
20 levels, indicating that insulin-induced down-regulation of GLUT4 at the PM did not account for the limited HA-GLUT4 labeling during the continuous antibody uptake.

The recycling kinetics of HA-GLUT4 was studied at different stages throughout fibroblast differentiation (Fig. 7). In parallel, antibody uptake was analysed by
25 immunofluorescence confocal microscopy (Fig. 7, left microscopy panels) as well as endogenous GLUT4 labeling and lipid droplet content in non-infected cells (Fig. 7, right microscopy panels).

There was a progressive decline in antibody uptake between days 0 and 4 of
30 differentiation. Expression of endogenous GLUT4 and lipid droplet formation were initially detected at day 3 when antibody uptake by non-stimulated cells had already decreased by 85% (compared with 100% at day 4). The final reduction in basal anti-HA uptake, between day 3 and 4, coincided with a massive growth of the cells (Fig. 7, right bottom microscopy panels).

35

The results attained suggest that only part of the intracellular GLUT4 pool may be released into the cell surface recycling system as opposed to reduced trafficking kinetics of the entire intracellular GLUT4 pool. To test this recycling studies were performed at different doses of insulin (Fig. 8). These studies revealed that the size of the recycling pool of GLUT4 was incrementally increased with increasing doses of insulin.

This phenomenon was evident for both GLUT4 WT and GLUT4 TAIL, although insulin had a less profound effect on GLUT4 TAIL due to its elevated levels in the recycling pathway in the basal state (Fig. 5 and 8B). Measurement of cell surface levels of HA-GLUT4 at the different insulin doses revealed that the insulin dose response curves for translocation of both GLUT4 WT and TAIL were similar, despite major differences in their basal recycling properties (Fig. 8B).

To rule out the possibility that this incremental effect of insulin on entry of GLUT4 into the cell surface recycling system might reflect intrinsic differences in insulin sensitivity between individual cells within the culture the dose response relationship in antibody uptake in individual cells using immunofluorescence microscopy was examined. As indicated in Fig. 8C the response among different cells was highly homogeneous such that at low doses of insulin most cells exhibited a low level of antibody uptake and at higher doses there was a uniform rather than a heterogeneous increase in antibody uptake.

25

EXAMPLE 5

Development of a high-throughput assay for determining GLUT4 translocation

To determine the efficacy of a high throughput assay for analysing the level of translocation of a labeled membrane transport protein HA-GLUT4 expressing 3T3-L1 adipocytes were grown in 384 well plates or first grown in Petri dishes and then relocated into the 384 well plates. An incubation period of 2 hours was observed after which 200nM insulin exposure was used for the indicated periods of time. For each time point the percentage of labeled GLUT4 (compared to the level of labeled GLUT4 following cell permeabilization) at the plasma membrane was calculated. As shown in Figure 9 approximately equal levels of GLUT4 translocation was observed in both

sample types. Accordingly, these results show the efficacy of a 384 high-throughput method for analysing GLUT4 translocation.

EXAMPLE 6

5 The effect of amino acid concentration on GLUT4 translocation

HA-GLUT4 expressing adipocytes were serum starved for 2 hours in Krebs Ringer Phosphate (KRP) buffer or in the same buffer supplemented with amino acid concentrations used in Dulbecco's modified eagle medium of Gibco (2x amino acids) or with half of the amino acid concentration (1x amino acids) respectively. Cells were then stimulated with 200nM insulin essentially as described above and the percentage of HA-GLUT4 WT translocated to the membrane determined as described *supra*. As shown in Fig. 10 the concentration of amino acids in the medium in which cells were incubated influenced the level of GLUT4 translocated to the plasma membrane.

15

EXAMPLE 7

Inducing GLUT4 translocation to the plasma membrane

3T3-L1 adipocytes expressing HA-GLUT4 WT were serum starved for 2 hours at 37°C. Following 20 minutes insulin stimulation with 200nM insulin, cells were incubated for additional 2 hours in serum free medium supplemented with 0.2% BSA and 0.3 or 0.6M sucrose. After post-fixation anti-HA immunolabeling the level of cell surface HA-GLUT4 levels was determined as a percentage of total HA-GLUT4 detected after cell lysis. As shown in Fig. 11, sucrose dramatically increases the level of HA-GLUT4 translocated to the plasma membrane of a cell. Furthermore, increasing concentrations of sucrose induce more GLUT4 to translocate to the plasma membrane in the presence of reduced levels of insulin.

25

EXAMPLE 8

30 Development of a model of insulin resistance

3T3-L1 adipocytes retrovirally infected with GLUT4 (described in Example 1) were incubated 24 hours or 48 hours either with 600nM insulin or with medium alone. After this chronic insulin stimulation (as indicated in Figure 10) at 37°C in a CO₂ incubator, cells were washed and 200 nM insulin was added for additional 10 or 30 minutes. Cell surface levels of HA-GLUT4 were measured using the fluorescence based assay

35

described *supra* and expressed as a percentage of total HA-GLUT4 detected in the cell. The experiment was also performed with the HA-GLUT4 TAIL mutant.

As shown in Figure 12A the level of GLUT4 at the plasma membrane of cells
5 incubated in the presence of serum was dramatically increased following 24h incubation in the presence of insulin. However, this effect was suppressed following 48h incubation in the presence of insulin.

A dramatically different effect was observed in cells incubated in the absence of serum
10 (either -serum or KRP). The levels of GLUT4 translocation observed were little more than basal levels (i.e. cells in the absence of insulin). These results indicate that the cells were resistant to insulin induced GLUT 4 translocation. This assay represents an attractive model of insulin resistance for, for example, screening for agents for treating disorders characterised by insulin resistance.

15 As shown in Figure 12B similar results were attained with the HA-GLUT4 TAIL mutant.

Furthermore, as shown in Figure 13 wortmannin was shown to have little effect on the
20 translocation of HA-GLUT4 in the presence of serum either following an acute or chronic exposure to insulin. HA-GLUT4 expressing 3T3-L1 adipocytes were grown in 96 well plates, incubated for 2 hours or overnight in medium supplemented with 10% fetal calf serum or no serum. 200nM insulin in case of acute stimulation and 600nM insulin in case of chronic stimulation have been used. After overnight stimulation cells
25 were washed and 200nM fresh insulin was added for 10 or 30 min.

However, following an acute exposure to insulin, wortmannin was able to reduce levels of HA-GLUT4 translocation in cells incubated in the absence of insulin. Following a chronic exposure of the cells to insulin wortmannin did not appear to significantly alter
30 the levels of GLUT4 translocated to the plasma membrane.

EXAMPLE 9

Screening a natural product library to determine an enhancer of GLUT4 translocation

35 HA-GLUT4 expressing 3T3-L1 adipocytes are grown in 384 well plates essentially as described in Example 5. Cells are then incubated 24 hours with 600nM insulin in the

absence of serum. After this chronic insulin stimulation at 37°C in a CO₂ incubator cells are incubated in the presence of a compound from a natural product library, such as, for example, the plant extract library from TimTec (Newark, USA). 200 nM insulin is then added for an additional 10 or 30 minutes to each well. Cell surface levels of
5 HA-GLUT4 is measured using the fluorescence based assay described *supra* and expressed as a percentage of total HA-GLUT4 detected in the cell. Results are also normalized for cell number using WGA, essentially as described in Example 2.

Samples are analysed to determine those natural products that are capable of inducing
10 HA-GLUT4 translocation to the plasma membrane to a degree similar to that observed in a cell incubated in the presence of both serum and insulin (i.e. a positive control).

Cells cultured in parallel are also assayed using trypan blue exclusion to determine those natural products that are toxic to cells. Following incubation of the cells in the
15 presence or absence (control) of the natural products, cells are treated with 1% trypan blue. The number of cells that have taken up the trypan blue stain in each treatment group is expressed as a percentage of the number of cells that have taken up the trypan blue stain in the control samples. Those compounds that significantly reduce the number of viable cells are considered to be at least partially toxic to a cell.

20

Compounds that enhance GLUT4 translocation without significantly reducing viability are then assessed using the assays *supra* to determine the concentration at which translocation is maximally enhanced without affecting cell viability.

25

EXAMPLE 10

In vivo analysis of an enhancer of GLUT4 translocation

Male C57BL/KS-Lep^{db} (*db/db*) and nondiabetic littermate mice (The Jackson Laboratory) are obtained at 7-8 weeks of age and housed in 12 hr of light per day at 21-
30 23°C and 40-60% humidity. All experiments begin at 10 weeks of age. A compound determined in Example 9 is administered by sub cutaneous injection. For glucose tolerance testing, all animals were fasted for 16-18 hr before gavaging with a standard glucose bolus, as outlined Tonra *et al.*, *Diabetes* 48: 588-594, 1999. Animals are then anesthetized and a bolus of insulin (1 unit) administered through the jugular vein; 2 or
35 10 min later, the liver is rapidly removed and frozen at -80°C until processed.

- Serum samples are taken between 1000 and 1200 hours and analyzed for glucose, triglycerides, and cholesterol with the Monarch blood chemistry analyzer (Instrumentation Laboratory, Lexington, MA). NEFA are analyzed with a diagnostic kit (Wako Chemical, Osaka) and insulin levels by ELISA (Linco Research Immunoassay, St. Charles, MO). For analysis of endogenous lipids, frozen sections of liver are mounted on glass slides and stained with oil red O. Liver glycogen is measured from frozen tissue by assaying for glucose after amyloglucosidase digestion with a correction for nonglycogen glucose (Tonra *et al.*, *Diabetes* 48: 588-594, 1999).
- 10 Using these assays, mice are then assessed to determine hyperinsulinemia, hyperglycemia and glucose tolerance essentially as described in Sleeman *et al.*, *Proc Natl Acad Sci U S A.* 100:14297-14302, 2003. For example, serum glucose and insulin levels are determined.

15 **EXAMPLE 11**

An assay to determine a suppressor of GLUT4 translocation

- HA-GLUT4 expressing 3T3-L1 adipocytes are grown in 384 well plates essentially as described in Example 5. Cells are then incubated with a compound from the natural product library *supra* and then 200nM insulin. The level of HA-GLUT4 translocated to the plasma membrane is then measured.
- 20

- Briefly, cells are fixed in 3% formaldehyde. After quenching with 50 mM glycine, cells are incubated for 20 min with 5% normal swine serum (NSS) in the absence or presence of 0.1% saponin to analyse the level of GLUT4 at the plasma membrane (PM) or the total cellular GLUT4 content, respectively. Cells are incubated for 60 min with a saturating concentration of either an antibody directed against the HA tag or a control non-relevant antibody (mouse IgG MOPC21) in PBS containing 2% NSS. After extensive washing, the cells are incubated for 20 min with 5% NSS in the presence or absence of 0.1% saponin to permeabilize all cells. Cells are incubated for 60 min with saturating concentrations of ALEXA488-conjugated goat-anti-mouse antibody (20 µg/ml) and ALEXA594-conjugated WGA (10 µg/ml) in PBS containing 2% NSS. After washing, fluorescence (emm 485/exc 520 and emm 544/exc 630) is measured using the bottom-reading mode in a fluorescence microtiter plate reader (FLUOstar Galaxy, BMG Labtechnologies, Offenburg, Germany). The percentage of GLUT4 at
- 25
- 30
- 35

the PM is calculated for each compound. ALEXA594-WGA fluorescence was used to correct for variation in cell density in each well.

As a positive control the K⁺/H⁺ exchanger, nigericin, is used. Nigericin is known to inhibit insulin mediated GLUT4 translocation Chu *et al.*, *J Cell Biochem.* 2002;85:83-91. The level of translocation of HA-GLUT4 for each natural compound is compared to that for nigericin and compounds with equal or greater inhibitory activity are selected.

In parallel cultures, the toxicity of each of the natural products is also assessed. Cell viability for each of the compounds tested is assessed using the CellTiter-Glo[®] Luminescent Cell Viability Assay (Promega) essentially according to manufacturer's instructions. Compounds that do not significantly reduce cell viability are selected for further analysis.

The compounds selected are then screened using the HA-GLUT4 translocation assay and the CellTiter-Glo[®] Luminescent Cell Viability Assay to determine the concentration at which each compound shows maximum activity without significantly reducing cell viability.

EXAMPLE 12

A model for GLUT1 translocation

12.1 Vector construction

A human GLUT1 cDNA containing an Hemagglutinin epitope tag in its first exofacial loop was kindly provided in the pCIS2 expression vector by the Al-Hasani Lab.

HA-GLUT1 is then excised from this pCIS2 vector by *Nde*I and *Kpn*I digestion and subcloned into the pOK12 plasmid. Following digestion with *Nde*I and *Kpn*I, this reporter GLUT1 gene tagged with HA is then excised from pOK12 plasmid as a 1.8 kb *Cla*I/*Xba*I fragment and subcloned into pBluescript plasmid digested with *Cla*I and *Xba*I. Following subcloning, the HA-Glut1 fragment is excised from pBluescript by *Bst*XI and *Sal*I digestion and directionally cloned into pBABE retrovirus expression vector digested with *Bst*XI and *Sal*I, thus generating the HA-GLUT1..

12.2 retrovirus production and transduction

Retroviral stocks of the construct is produced using the method described in Pear *et al. Proc. Natl Acad. Sci. U.S.A.* 90: 8392-8396 1993. To generate C2C12 myoblast cells stably expressing the expression construct C2C12 were infected with the relevant virus for 3-5h in the presence of 4µg/ml Polybrene (Sigma). After a 48h recovery period, 5 infected cells are then selected in DMEM containing 10% FCS and supplemented with 2µg/ml puromycin (Sigma).

Transduced myoblasts are seeded in proliferation medium (Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% FCS) at a density of 12,000 cells 10 per cm² and grown for 48 h to confluency. Cells are washed once with serum-free medium and induced to fuse in medium containing 2% horse serum (differentiation medium).

12.3 Analysis of translocation of HA-GLUT1 in differentiated C2C12 cells

15 Retrovirally-transduced differentiated C2C12 cells expressing HA-tagged GLUT1 are subcultured for 30 hours. Insulin is then added at different time points, after which the cells are fixed in 3% formaldehyde. After quenching with 50 mM glycine, cells are incubated for 20 min with 5% normal swine serum (NSS) in the absence or presence of 0.1% saponin to analyse the level of HA-GLUT1 at the plasma membrane (PM) or the 20 total cellular HA-GLUT1 content, respectively. Cells are incubated for 60 min with a saturating concentration of either an antibody directed against the HA tag or a control non-relevant antibody (mouse IgG MOPC21) in PBS containing 2% NSS. After extensive washing, the cells are incubated for 20 min with 5% NSS in the presence or absence of 0.1% saponin to permeabilize the cells. Cells are incubated for 60 min with 25 saturating concentrations of ALEXA488-conjugated goat-anti-mouse antibody (20 µg/ml) and ALEXA594-conjugated WGA (10 µg/ml) in PBS containing 2% NSS. After washing, fluorescence (emm 485/exc 520 and emm 544/exc 630) is measured using the bottom-reading mode in a fluorescence microtiter plate reader (FLUOstar Galaxy, BMG Labtechnologies, Offenburg, Germany). The percentage of GLUT1 at 30 the PM is calculated for each condition. ALEXA594-WGA fluorescence was used to correct for variation in cell density in each well.

As a positive control a sample of cells are also incubated in the presence of Dehydroepiandrosterone (DHEA). DHEA has been previously shown to enhance 35 levels of GLUT1 at the plasma membrane of a cell (Perrini *et al., Diabetes* 53:41-52, 2004).

EXAMPLE 13

A model to determine the effect of a CFTR mutation on CFTR translocation

5 The coding region of the CFTR gene (SEQ ID NO: 35) is isolated using methods essentially as described in Rommens *et al.*, *Proc. Natl. Acad. Sci. USA* 88: 7500-7504, 1990. A double stranded oligonucleotide encoding HA tag is then inserted so as to encode the tag at the N terminus of the protein. The N-terminus of the CFTR is predicted to be an extracellular domain of the protein.

10

A vector comprising nucleic acid encoding the $\Delta F508$ mutant of CFTR (SEQ ID NO: 62) is produced essentially as described in Tabacharani *et al.*, *Nature*, 352: 628-632, 1991. The nucleic acid encoding the mutant CFTR is then modified to insert a double stranded oligonucleotide encoding HA tag is then inserted so as to encode the tag at the

15 N terminus of the protein.

Each of the modified constructs is then cloned into the pBABE retroviral vector.

20 Retroviral stocks of each of the constructs are then produced using the method described in Pear *et al.* *Proc. Natl Acad. Sci. U.S.A.* 90: 8392-8396 1993. To generate COS cells stably expressing the expression construct COS were infected with the relevant virus for 3-5h in the presence of 4 μ g/ml Polybrene (Sigma). After a 48h recovery period, infected cells are then selected in DMEM containing 10% FCS and supplemented with 2 μ g/ml puromycin (Sigma).

25

The level of plasma membrane associated HA-CFTR or HA-CFTR- $\Delta F508$ is then determined. Briefly, Retrovirally-transduced cells expressing HA-tagged CFTR or CFTR- $\Delta F508$ are subcultured for 30 hours. Cells are then fixed in 3% formaldehyde. After quenching with 50 mM glycine, cells are incubated for 20 min with 5% normal
30 swine serum (NSS) in the absence or presence of 0.1% saponin to analyse the level of HA-labeled CFTR or mutant thereof at the plasma membrane (PM) or the total cellular HA-CFTR or CFTR- $\Delta F508$ content, respectively. Cells are incubated for 60 min with a saturating concentration of either an antibody directed against the HA tag or a control non-relevant antibody in PBS containing 2% NSS. After extensive washing, the cells
35 are incubated for 20 min with 5% NSS in the presence or absence of 0.1% saponin to permeabilize the cells. Cells are incubated for 60 min with saturating concentrations of

ALEXA488-conjugated goat-anti-mouse antibody (20 µg/ml) and ALEXA594-conjugated WGA (10 µg/ml) in PBS containing 2% NSS. After washing, fluorescence (emm 485/exc 520 and emm 544/exc 630) is measured using the bottom-reading mode in a fluorescence microtiter plate reader (FLUOstar Galaxy, BMG Labtechnologies, 5 Offenburg, Germany). The percentage of CFTR or CFTR-ΔF508 at the PM is calculated for each condition. ALEXA594-WGA fluorescence was used to correct for variation in cell density in each well.

By comparing the level of HA-CFTR at the plasma membrane compared to the level of 10 HA-CFTR-ΔF508 translocated to the plasma membrane, the effect of the ΔF508 mutation on translocation is determined.

We claim:

1. A process for determining the level of a membrane transport protein translocated to the plasma membrane of a cell, said method comprising:
 - (a) determining the level of a membrane transport protein at the plasma membrane of the cell using a method comprising:
 - (i) contacting the cell with a ligand that binds to an extracellular domain of the membrane transport protein for a time and under conditions sufficient for the ligand to bind to the membrane transport protein at the plasma membrane of the cell; and
 - (ii) determining the level of ligand bound to the membrane transport protein;
 - (b)
 - (i) permeabilizing or disrupting the plasma membrane of a cell and contacting the membrane transport protein within the cell with the ligand for a time and under conditions sufficient for the ligand to bind to the membrane transport protein; and
 - (ii) determining the level of ligand bound to the membrane transport protein; and
 - (c) comparing the level of ligand determined at (a) (ii) and (b) (ii) to determine the level of the membrane transport protein at the plasma membrane relative to the level of the membrane transport protein inside the cell.
2. The process according to claim 1 wherein the membrane transport protein is a glucose transport (GLUT) protein.
3. The process according to claim 2 wherein the membrane transport protein is GLUT4.
4. The process according to claim 3 wherein the GLUT4 comprises an amino acid sequence at least 80% identical to the amino acid sequence set forth in SEQ ID NO: 2.
5. The process according to claim 2 wherein the membrane transport protein is GLUT1.

6. The process according to claim 5 wherein the GLUT1 comprises an amino acid sequence at least 80% identical to the amino acid sequence set forth in SEQ ID NO: 12.
7. The process according to claim 1 wherein the membrane transport protein is a mutant membrane transport protein having a reduced rate of recycling or transporter internalization compared to a wild-type form of the membrane transport protein.
8. The process according to claim 7 wherein the reduced rate of recycling or transporter internalization of the mutant membrane transport protein increases the level of the mutant membrane transport protein at the plasma membrane of a cell compared to the level of a wild-type form of the membrane transport protein.
9. The process according to claim 8 wherein the mutant protein is a mutant GLUT4 protein.
10. The process according to claim 10 wherein the mutant GLUT4 protein comprises an amino acid sequence at least 80% identical to an amino acid sequence selected from the group consisting of SEQ ID NO: 5, SEQ ID NO: 7 and SEQ ID NO: 9.
11. The process according to claim 1 wherein the membrane transport protein is labeled to facilitate binding of the ligand to the membrane transport protein.
12. The process according to claim 11 wherein the label comprises one or more copies of a peptide, polypeptide or protein that is heterologous to the membrane transport protein.
13. The process according to claim 12 wherein the label comprises one or more copies of a peptide, polypeptide or protein selected from the group consisting of influenza virus hemagglutinin (HA) (SEQ ID NO: 15), Simian Virus 5 (V5) (SEQ ID NO: 16), polyhistidine (SEQ ID NO: 17), c-myc (SEQ ID NO: 18), FLAG (SEQ ID NO: 19), GST (SEQ ID NO: 22), MBP (SEQ ID NO: 23), GAL4 (SEQ ID NO: 24), β -galactosidase (SEQ ID NO: 25), enhanced green fluorescence protein (eGFP) (SEQ ID NO: 26), yellow fluorescent protein (SEQ ID NO: 27), soluble modified blue fluorescent protein (SEQ ID NO: 28), soluble-modified red-

shifted green fluorescent protein (SEQ ID NO: 29), cyan fluorescent protein (SEQ ID NO: 30), biotin, streptavidin, a peptide comprising the amino acid sequence set forth in SEQ ID NO: 20, a peptide comprising the amino acid sequence set forth in SEQ ID NO: 21, a peptide comprising the amino acid sequence set forth in SEQ ID NO: 31 and mixtures thereof.

14. The process according to claim 13 wherein the label comprises influenza virus hemagglutinin (HA) (SEQ ID NO: 15).
15. The process according to claim 12 wherein the label is positioned within an extracellular domain of the membrane transport protein.
16. The process according to claim 15 wherein the label is positioned within the first extracellular domain of a GLUT protein or a mutant thereof.
17. The process according to claim 12 wherein the labeled membrane transport protein is a GLUT4 protein or a mutant GLUT4 protein that comprises an amino acid sequence at least 80% identical to an amino acid sequence selected from the group consisting of SEQ ID NO 4, SEQ ID NO: 6, SEQ ID NO: 8 and SEQ ID NO: 10.
18. The process according to claim 12 wherein the labeled membrane transport protein is a GLUT1 protein that comprises an amino acid sequence at least 80% identical to the amino acid sequence set forth in SEQ ID NO: 13.
19. The process according to claim 1 wherein the cell is a eukaryotic cell.
20. The process according to claim 19 wherein the cell is a mammalian cell
21. The process according to claim 20 wherein the cell is a cell selected from the group consisting of a 3T3-L1 fibroblast cell, a 3T3-L1 adipocyte cell and a C2C12 cell.
22. The process according to claim 1 wherein the ligand capable of binding to the membrane transport protein is an antibody.

23. The process according to claim 22 wherein the antibody is a monoclonal antibody.
24. The process according to claim 23 wherein the monoclonal antibody is an anti-hemagglutinin (HA) tag antibody capable of binding to an amino acid sequence set forth in SEQ ID NO: 15.
25. The process according to any one of claims 22 to 24 wherein the antibody is labeled with a detectable marker selected from the group consisting of an enzyme label, a radiolabel and a fluorescent label.
26. The process according to any one of claims 23 to 25 wherein the antibody is labeled with a fluorescent label.
27. The process according to claim 1 wherein the plasma membrane is permeabilized or disrupted by contacting the plasma membrane with an agent that permeabilizes or disrupts a membrane for a time and under conditions sufficient for permeabilization or disruption to occur.
28. The process according to claim 27 wherein the agent that permeabilizes or disrupts a membrane is selected from the group consisting of saponin, n-octyl-glucopyranoside, n-Dodecyl β -D-maltoside, N-Dodecanoyl-N-methylglycine sodium salt, hexadecyltrimethylammonium bromide, deoxycholate, a non-ionic detergent, streptolysin-O (SEQ ID NO: 32), α -hemolysin (SEQ ID NO: 33), tetanolysin (SEQ ID NO: 34) and mixtures thereof.
29. The process according to claim 28 wherein the agent that permeabilizes or disrupts the membrane is saponin.
30. The process according to claim 1 wherein the level of the ligand bound to the membrane transport protein is determined by a process comprising contacting the ligand with an antibody that specifically binds to the ligand for a time and under conditions sufficient for an antibody-antigen complex to form and determining the level of the complex wherein the level of the complex indicates the level of the ligand bound to the membrane transport protein.

31. The process according to claim 1 or 30 wherein the level of the ligand bound to the membrane transport protein is determined using an assay selected from the group consisting of immunofluorescence, immunohistochemistry, and an immunosorbent assay.
32. The process according to claim 1 or 30 wherein the level of the ligand bound to the membrane transport protein is determined using a fluorescence linked immunosorbent assay.
33. The process according to claim 1 additionally comprising providing the cell expressing the membrane transport protein.
34. The process according to claim 33 wherein providing the cell expressing the membrane protein comprises transforming or transfecting the cell with an expression construct that encodes the membrane protein.
35. The process according to claim 1 additionally comprising fixing the cell.
36. The process according to claim 35 wherein the cell is fixed prior to or at the same time as permeabilizing or disrupting the plasma membrane of the cell.
37. The process according to claim 35 or 36 wherein the cell is fixed with a compound selected from the group consisting of formaldehyde, paraformaldehyde, alcohol, methanol and glutaraldehyde.
38. The process according to claim 35 or 36 wherein the cell is fixed with formaldehyde.
39. The process according to claim 1 additionally comprising inducing translocation of the membrane transport protein to the plasma membrane.
40. The process according to claim 39 wherein inducing translocation of the membrane transport protein to the plasma membrane comprises contacting the cell with an amount of one or more peptides, polypeptides, proteins or compounds sufficient to induce translocation of the membrane transport protein for a time and under conditions sufficient for translocation to occur.

41. The process according to claim 40 wherein the cell is contacted with an amount of sucrose and/or an amount of insulin sufficient to induce translocation.
42. The process according to claim 41 wherein the cell is contacted with sucrose and/or insulin in the presence of serum.
43. The process according to claim 1 additionally comprising inducing resistance to translocation of the membrane transport protein in the cell.
44. The process according to claim 43 wherein the membrane transport is a GLUT protein or a mutant GLUT protein and wherein inducing resistance to translocation of the membrane transport protein in the cell comprises contacting the cell with an amount of insulin sufficient to induce resistance to insulin induced translocation for a time and under conditions sufficient for resistance to insulin induced translocation to occur.
45. The process according to claim 44 wherein the cell is contacted with insulin in the absence of serum.
46. The process according to claim 45 wherein the cell is contacted with insulin for between about 24 hours and about 48 hours.
47. The process of claim 1 comprising:
 - (a) determining the level of the membrane transport protein at the plasma membrane of a cell using a method comprising:
 - (i) contacting a cell with a ligand that binds to an extracellular domain of the membrane transport protein for a time and under conditions sufficient for the ligand to bind to the membrane transport protein; and
 - (ii) determining the level of ligand bound to the membrane transport protein;
 - (b) determining the level of the membrane transport protein within another cell using a method comprising:
 - (i) permeabilizing or disrupting the other cell;

- (ii) contacting the membrane transport protein within the cell with the ligand for a time and under conditions sufficient for the ligand to bind the membrane transport protein;
 - (iii) determining the level of ligand bound to the membrane transport protein; and
 - (c) comparing the level of ligand detected at (a) (ii) and (b) (iii) to determine the level of the labeled membrane transport protein at the plasma membrane relative to the total level of labeled membrane transport protein.
48. The process according to claim 47 wherein the cells are isogenic or from the same cell line.
49. The process according to claim 47 or 48 wherein the cells are cultured under substantially similar conditions.
50. The process according to claim 49 wherein the level of the membrane transport protein at the plasma membrane of the cell and the level of membrane transport protein within the cell are each determined in a plurality of cells.
51. The process according to claim 50 additionally comprising normalizing the determined level of ligand bound to the membrane transport protein with regard to the number of cells in which the level of ligand bound to the membrane transport protein is determined.
52. The process according to claim 51 wherein the number of cells is determined by a method comprising contacting the cells with an antibody or ligand capable of binding to a cell or component thereof for a time and under conditions sufficient for binding of the antibody or ligand to the cell or component thereof and determining the level of antibody bound to the cells, wherein the level of antibody or ligand bound to the cells is indicative of the number of cells.
53. The process according to claim 52 wherein the ligand is wheat germ aggluti

internalization compared to a wild-type form of the membrane transport protein, said process comprising:

(a) determining the level of the labeled GLUT4 protein or labeled mutant GLUT4 protein at the plasma membrane of a cell expressing the labeled GLUT4 protein or labeled mutant GLUT4 protein using a method comprising:

- (i) contacting the cell with a ligand that binds to the label for a time and under conditions sufficient for the ligand to bind to the labeled GLUT4 protein or labeled mutant GLUT4 protein; and
- (ii) determining the level of ligand bound to the labeled GLUT4 protein or labeled mutant GLUT4 protein;

(b) determining the level of membrane transport protein within another cell expressing the labeled GLUT4 protein or labeled mutant GLUT4 protein using a method comprising:

- (i) permeabilizing or disrupting the other cell;
- (ii) contacting the labeled GLUT4 protein or labeled mutant GLUT4 protein within the cell with a ligand that binds to the label for a time and under conditions sufficient for the ligand to bind to the labeled GLUT4 protein or labeled mutant GLUT4 protein;
- (iii) determining the level of ligand bound to the labeled GLUT4 protein or labeled mutant GLUT4 protein; and

(c) comparing the level of ligand detected at (a) (ii) and (b) (iii) to determine the level of the labeled GLUT4 protein or labeled mutant GLUT4 protein at the plasma membrane relative to the total level of labeled GLUT4 protein or labeled mutant GLUT4 protein.

55. A process for determining the level of a labeled GLUT4 protein or a labeled mutant GLUT4 protein translocated to the plasma membrane of a cell that is resistant to insulin induced GLUT4 translocation, wherein said labeled mutant GLUT4 protein has a reduced rate of recycling or transporter internalization compared to a wild-type form of the membrane transport protein, said process comprising:

(a) contacting a plurality of cells expressing a labeled GLUT4 protein or a labeled mutant GLUT4 protein with an amount of insulin sufficient to induce resistance to insulin induced translocation for a time and under conditions sufficient to induce resistance to insulin induced GLUT4 translocation in the cell, wherein the cells are contacted with insulin in the absence of serum and wherein

the cells are contacted with insulin for a period of time from about 24 hours to about 48 hours;

(b) determining the level of the labeled GLUT4 protein or labeled mutant GLUT4 protein at the plasma membrane of a cell at (a) using a method comprising:

(i) contacting the cell with a ligand that binds to the label for a time and under conditions sufficient for the ligand to bind to the labeled GLUT4 protein or labeled mutant GLUT4 protein; and

(ii) determining the level of ligand bound to the labeled GLUT4 protein or labeled mutant GLUT4 protein;

(c) determining the level of labeled GLUT4 protein or labeled mutant GLUT4 protein in another cell at (a) but not (b) using a method comprising:

(i) permeabilizing or disrupting the other cell;

(ii) contacting the labeled GLUT4 protein or labeled mutant GLUT4 protein within the cell with a ligand that binds to the label for a time and under conditions sufficient for the ligand to bind to the labeled GLUT4 protein or labeled mutant GLUT4 protein;

(iii) determining the level of ligand bound to the labeled GLUT4 protein or labeled mutant GLUT4 protein; and

(d) comparing the level of ligand detected at (b) (ii) and (c) (iii) to determine the level of the labeled GLUT4 protein or labeled mutant GLUT4 protein at the plasma membrane relative to the total level of labeled GLUT4 protein or labeled mutant GLUT4 protein.

56. A process for determining the level of recycling of a membrane transport protein in a cell or a change in the level of recycling of a cell comprising:

(a) determining the level of the membrane transport protein translocated to the plasma membrane of a cell using the process according to any one of claims 1 to 54;

(b) determining the level of the membrane transport protein translocated to the plasma membrane of another cell using the process according to any one of claims 1 to 54, wherein the other cell is cultured for a longer period of time than the cell at (a); and

(c) comparing the level of the membrane transport protein translocated to the plasma membrane at (a) and (b) to thereby determine the level of recycling of the membrane transport protein in the cell, wherein a change in the level of the

membrane transport protein translocated to the plasma membrane indicates a change in the level of recycling of a membrane transport protein.

57. A process for determining a mutation in a nucleic acid encoding a mutant membrane transport protein that is capable of modulating translocation of said membrane transport protein, said method comprising:
- (i) determining the level of the mutant membrane transport protein translocated to the plasma membrane of a cell using the process according to any one of claims 1 to 54; and
 - (ii) determining the level of the wild-type form of the membrane transport protein translocated to the plasma membrane of a cell using the process according to any one of claims 1 to 54,
- wherein an enhanced or suppressed level of translocation of the membrane transport protein at (a) compared to (b) indicates that the nucleic acid comprises a mutation that is capable of modulating the level of level of translocation of the membrane transport protein to the plasma membrane.
58. A process for determining an agent that modulates translocation of a membrane transport protein to the plasma membrane of a cell, said process comprising:
- (a) determining the level of a membrane transport protein translocated to the plasma membrane of a cell in the absence of a candidate agent by performing the process according to any one of claims 1 to 54;
 - (b) determining the level of the membrane transport protein translocated to the plasma membrane of a cell in the presence of the candidate agent by performing the process according to any one of claims 1 to 54, wherein a difference in the level of the membrane transport protein translocated to the plasma membrane of a cell at (a) compared to (b) indicates that the candidate agent modulates translocation of the membrane transport protein.
 - (c) optionally, determining the structure of the candidate agent;
 - (d) optionally, providing the name or structure of the candidate agent; and
 - (e) optionally, providing, the candidate agent.
59. A process for determining a candidate compound for the treatment of insulin resistance comprising:
- (a) determining the level of the labeled GLUT4 protein or the labeled mutant GLUT4 protein translocated to the plasma membrane of a cell in the absence of a

- candidate agent by performing the process according to claim 55, wherein said labeled mutant GLUT4 protein has a reduced rate of recycling or transporter internalization compared to a wild-type form of the membrane transport protein; and
- (b) determining the level of the labeled GLUT4 protein or a labeled mutant GLUT4 protein translocated to the plasma membrane of another cell in the presence of the candidate agent by performing the process according to claim 55, wherein said labeled mutant GLUT4 protein has a reduced rate of recycling or transporter internalization compared to a wild-type form of the membrane transport protein and wherein a candidate agent that enhances the level of translocation of the labeled GLUT4 protein or a labeled mutant GLUT4 protein is a candidate agent for the treatment of insulin resistance.
 - (c) optionally, determining the structure of the candidate agent;
 - (d) optionally, providing the name or structure of the candidate agent; and
 - (e) optionally, providing, the candidate agent.
60. The process of claim 59 wherein the insulin resistance is associated with diabetes.
61. The process according to claim 60 wherein the diabetes is type II diabetes.
62. A process for manufacturing a medicament for the treatment of insulin resistance comprising:
- (a) determining a candidate compound for the treatment of insulin resistance using a process comprising:
 - (i) determining the level of the labeled GLUT4 protein or the labeled mutant GLUT4 protein translocated to the plasma membrane of a cell in the absence of a candidate agent by performing the process according to claim 55, wherein said labeled mutant GLUT4 protein has a reduced rate of recycling or transporter internalization compared to a wild-type form of the membrane transport protein; and
 - (ii) determining the level of the labeled GLUT4 protein or a labeled mutant GLUT4 protein translocated to the plasma membrane of another cell in the presence of the candidate agent by performing the process according to claim 55, wherein said labeled mutant GLUT4 protein has a reduced rate of recycling or transporter internalization compared to a wild-type form of the membrane transport protein and wherein a candidate agent that enhances the

level of translocation of the labeled GLUT4 protein or a labeled mutant GLUT4 protein is a candidate agent for the treatment of insulin resistance.

- (b) optionally, isolating the candidate agent;
- (c) optionally, providing the name or structure of the candidate agent;
- (d) optionally, providing the candidate agent; and
- (e) using the candidate agent in the manufacture of a medicament for the treatment of insulin resistance.

1/37

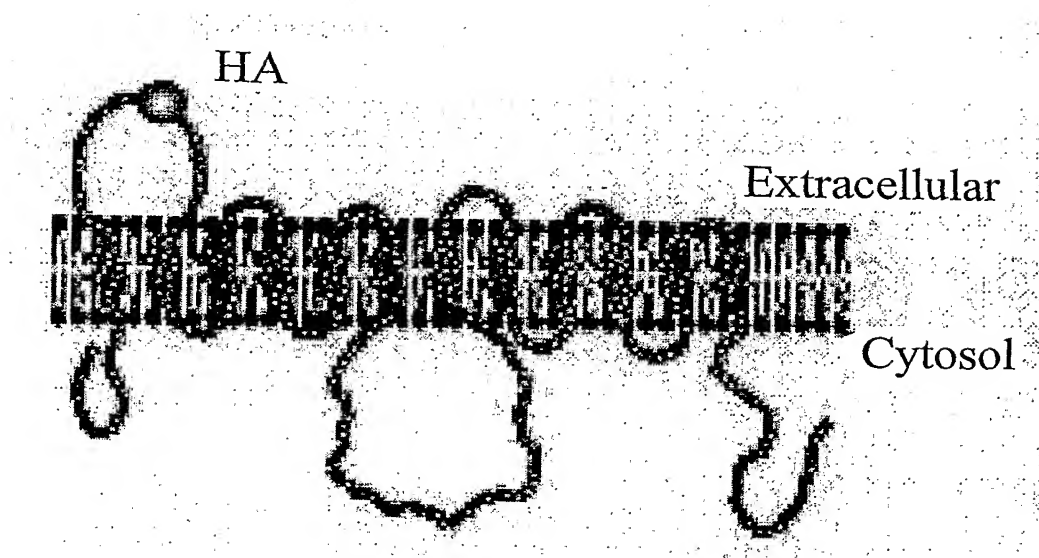


Figure 1a

2/37

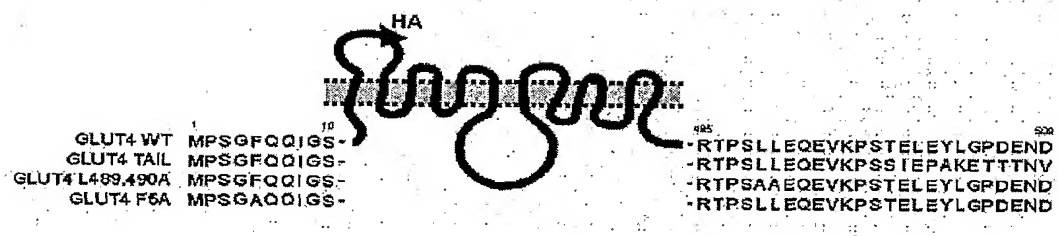


Figure 1b

3/37

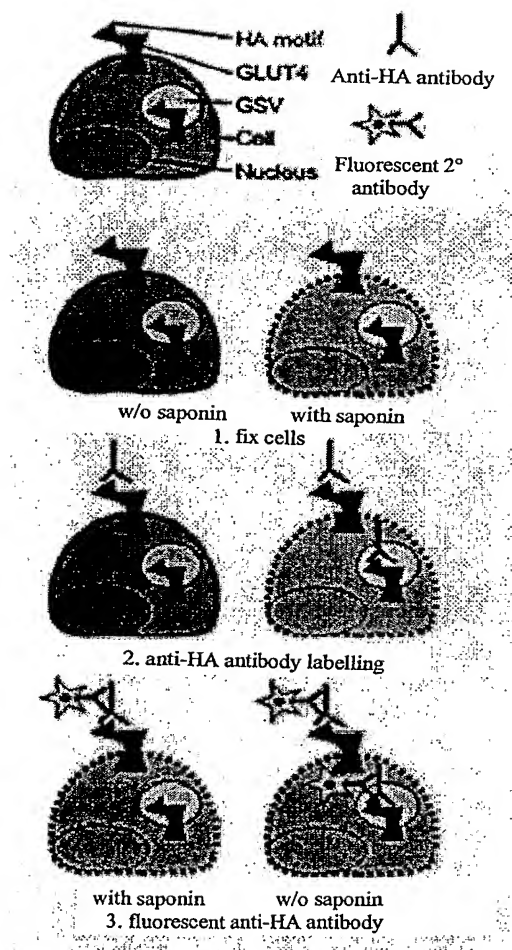


Figure 1c

4/37

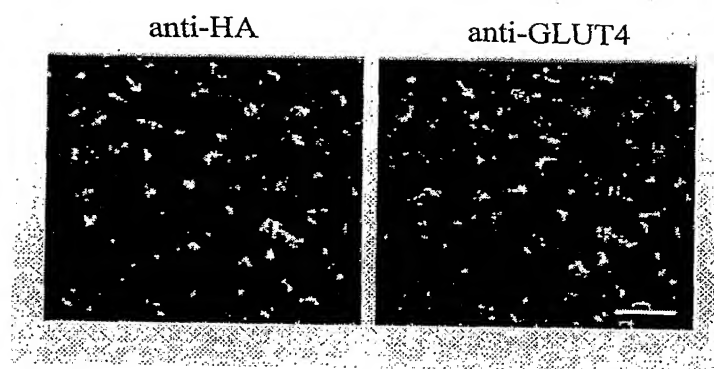


Figure 1d

5/37

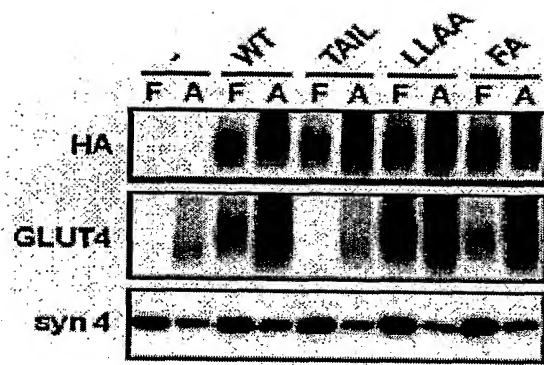


Figure 1e

6/37

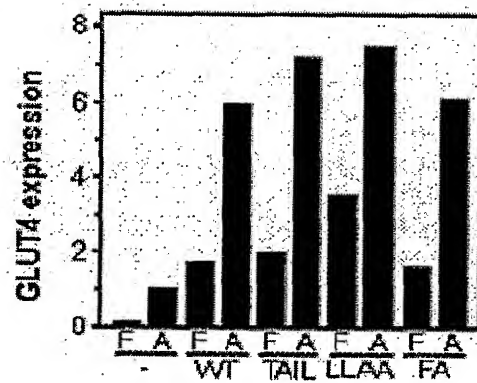


Figure 1f

7/37

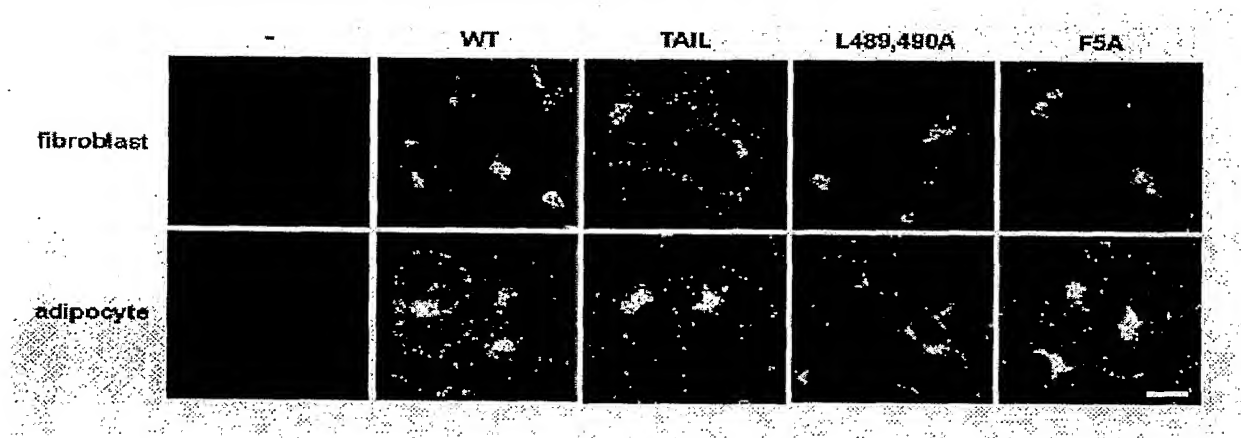


Figure 1g

8/37

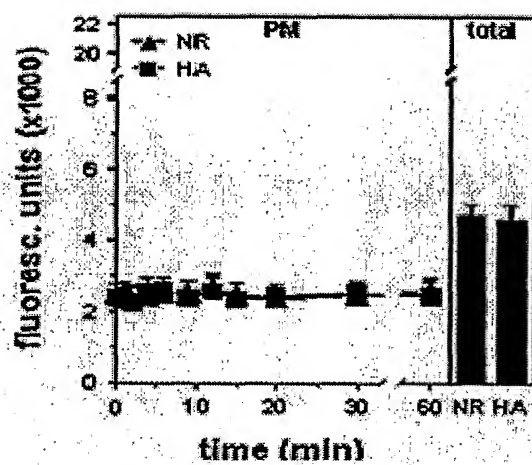


Figure 2a

9/37

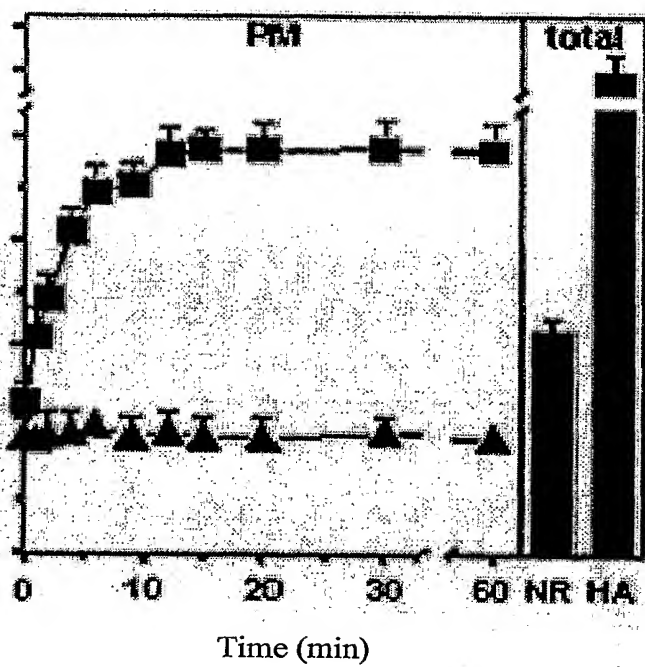


Figure 2b

10/37

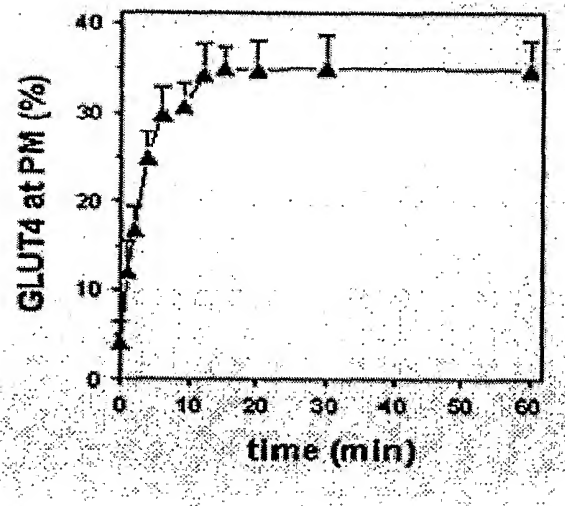


Figure 2c

11/37

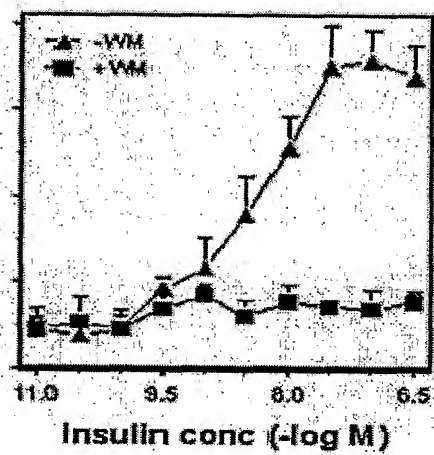


Figure 2d

12/37

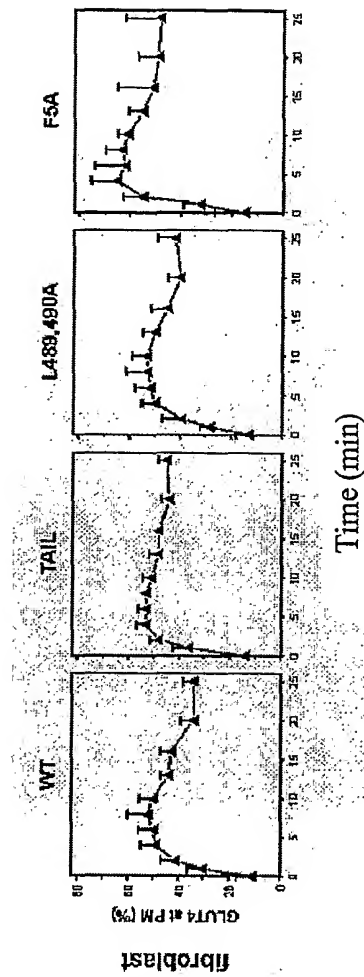


Figure 3a

13/37

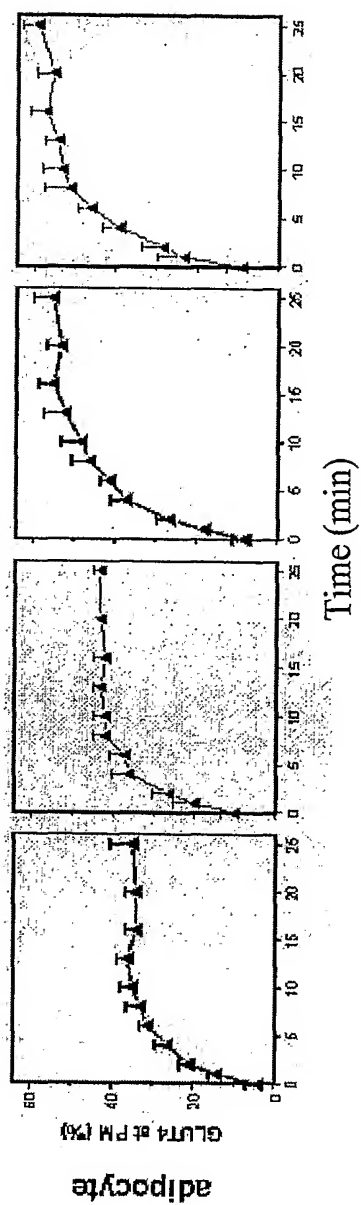


Figure 3b

14/37

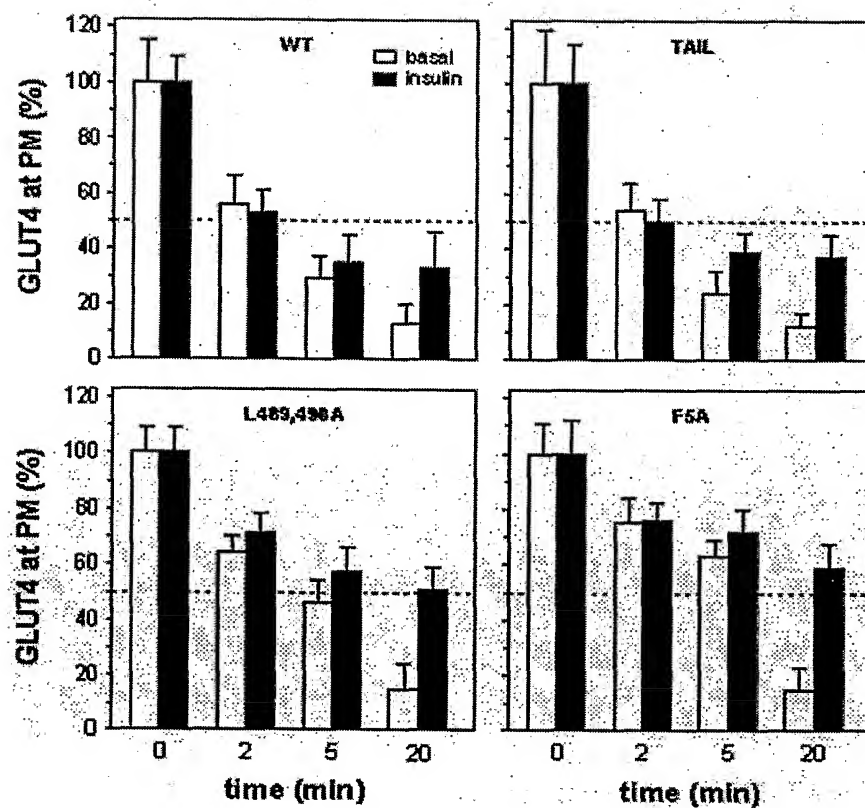


Figure 4

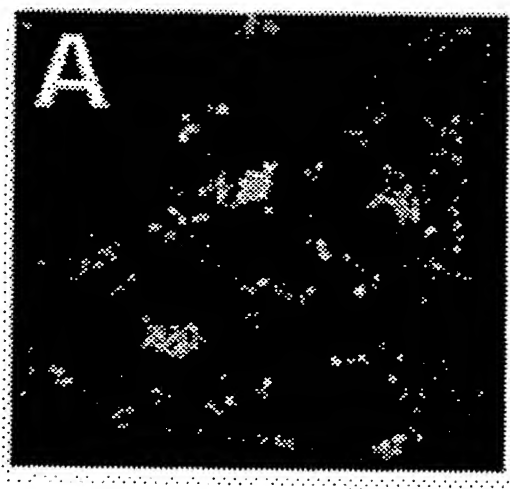


Figure 5a

16/37

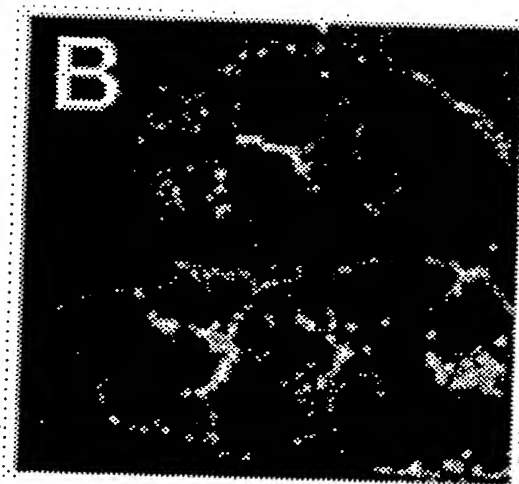


Figure 5b

17/37

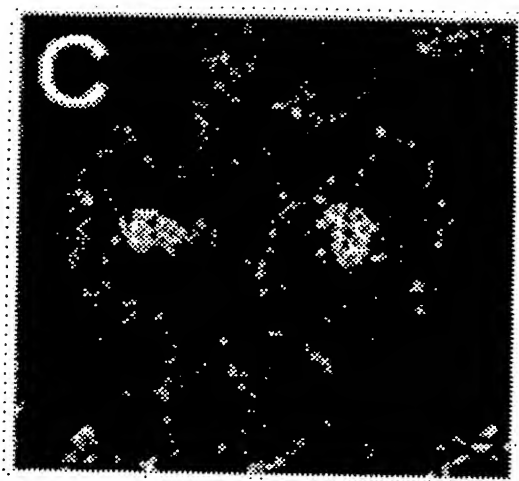


Figure 5c

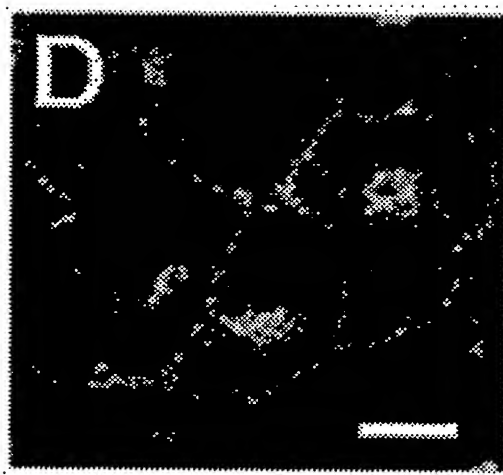


Figure 5d

19/37

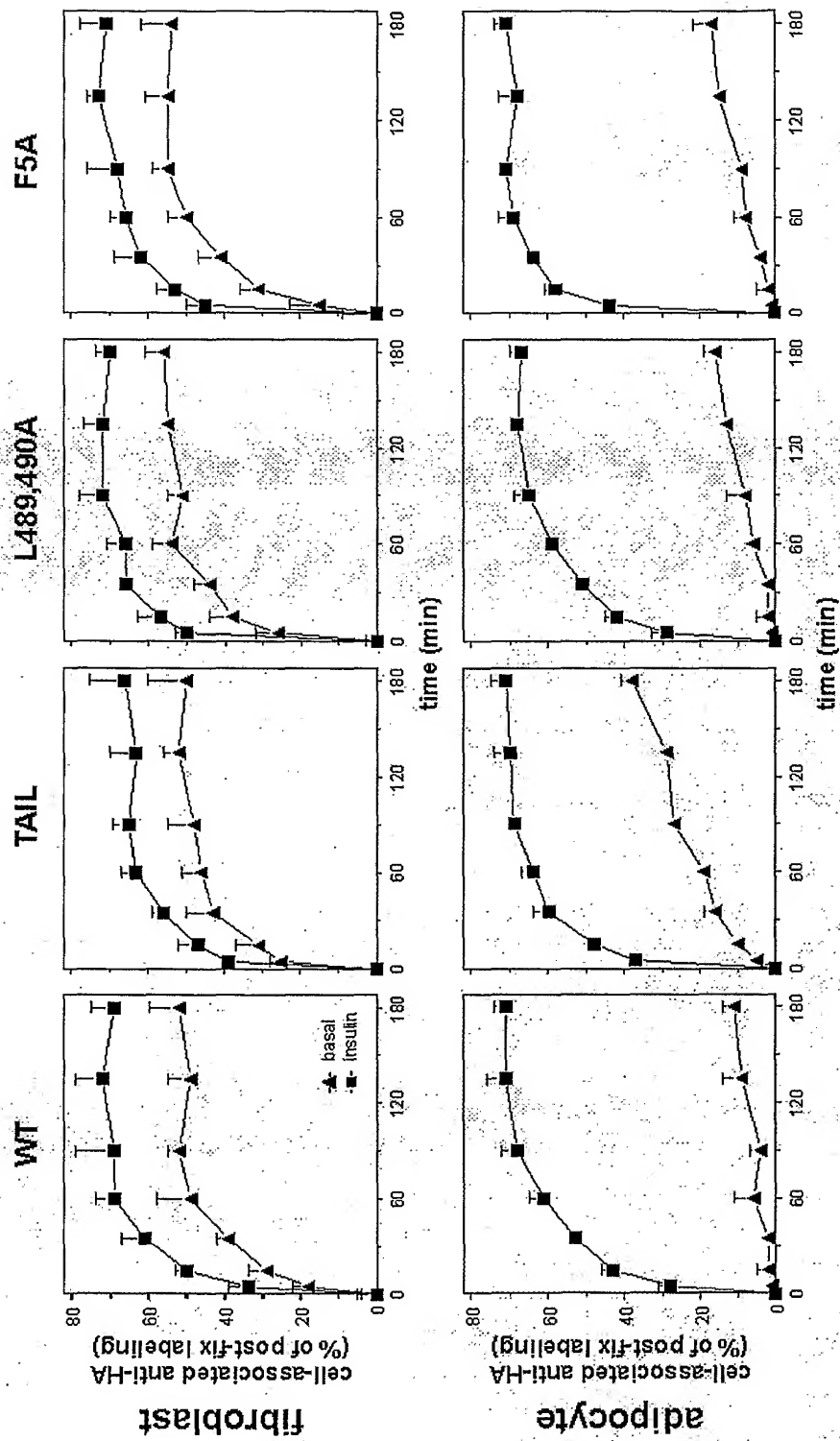


Figure 5e

20/37

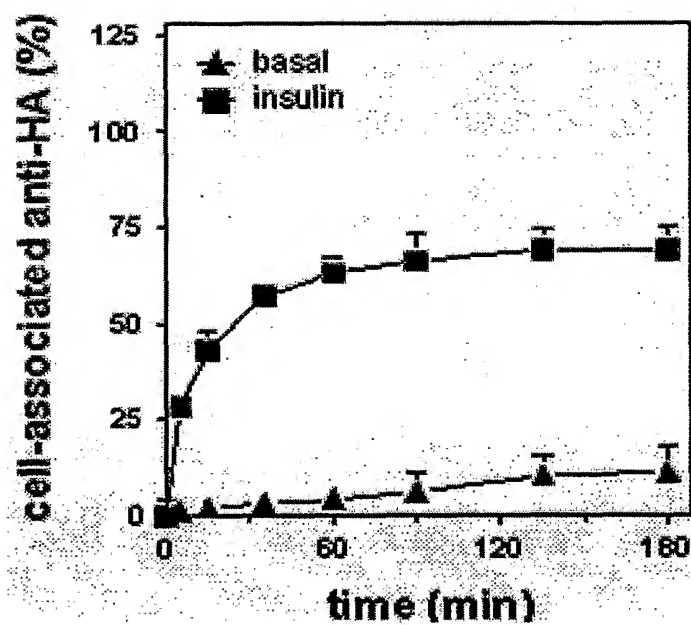


Figure 6a

21/37

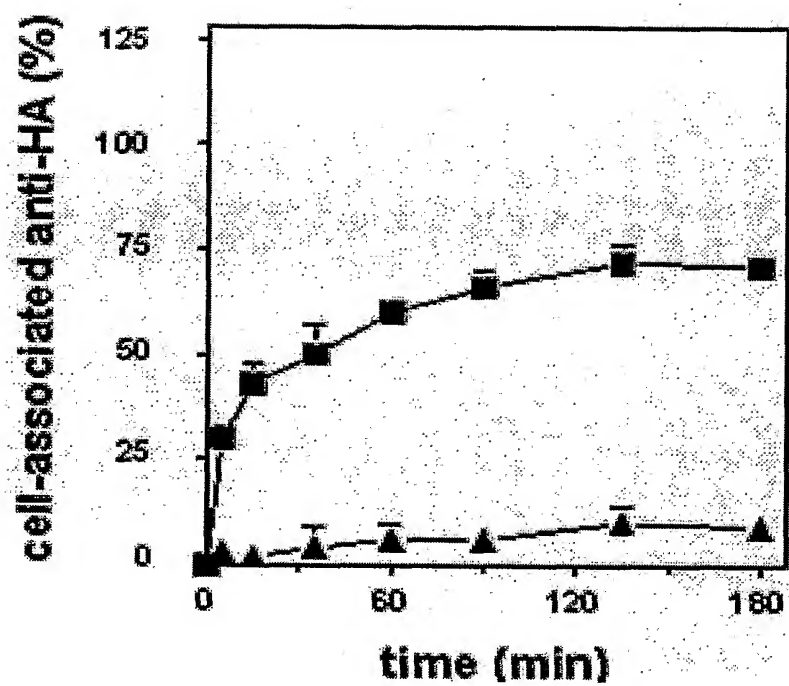


Figure 6b

22/37

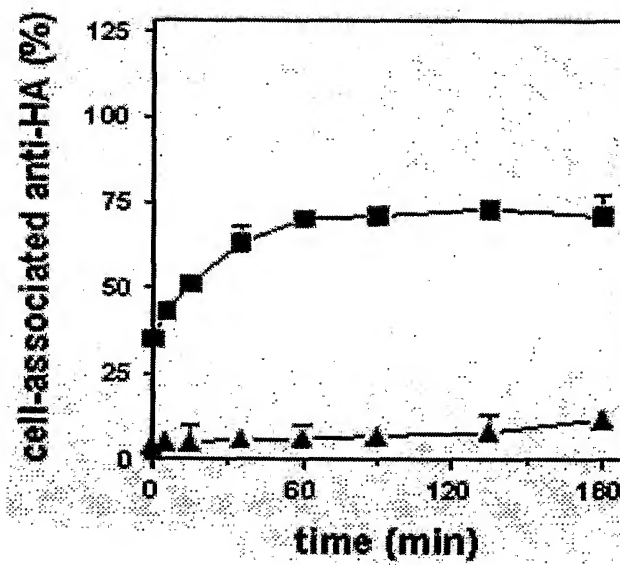


Figure 6c

23/37

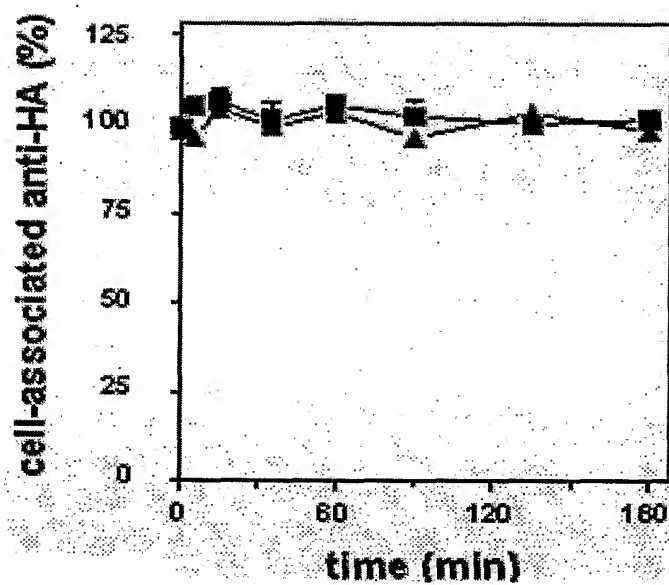


Figure 6d

24/37

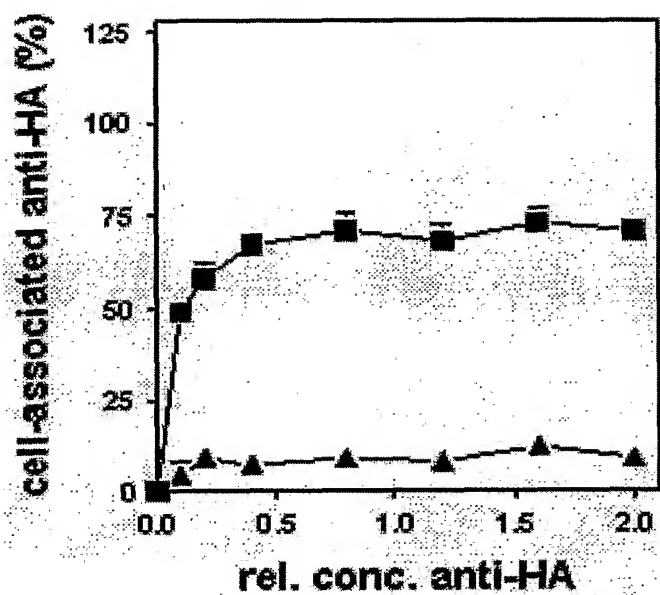


Figure 6e

25/37

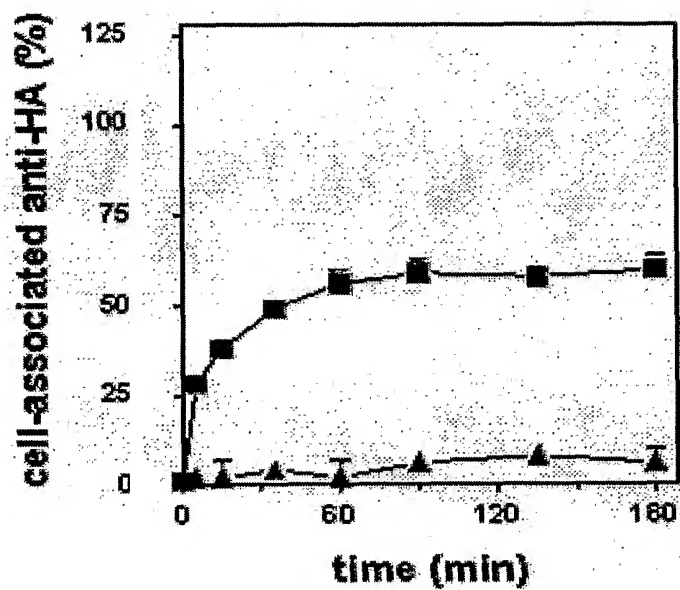


Figure 6f

26/37

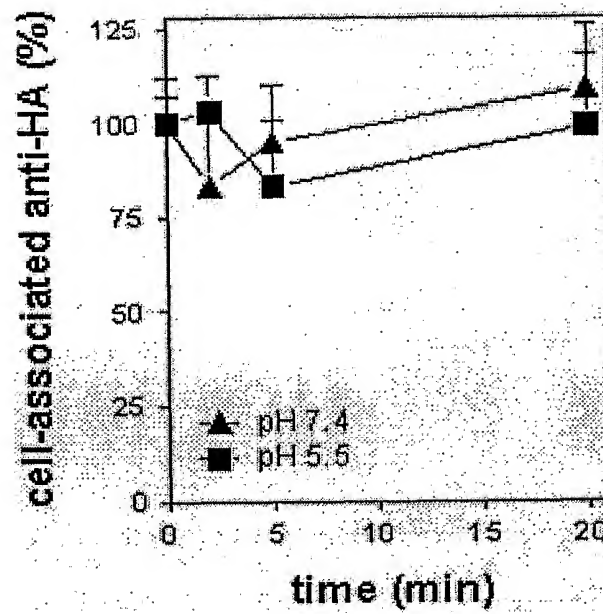


Figure 6g

27/37

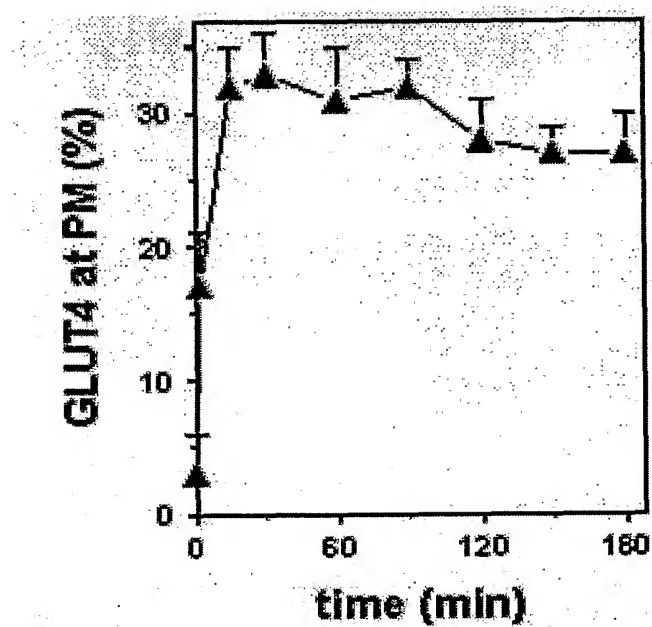


Figure 6h

28/37

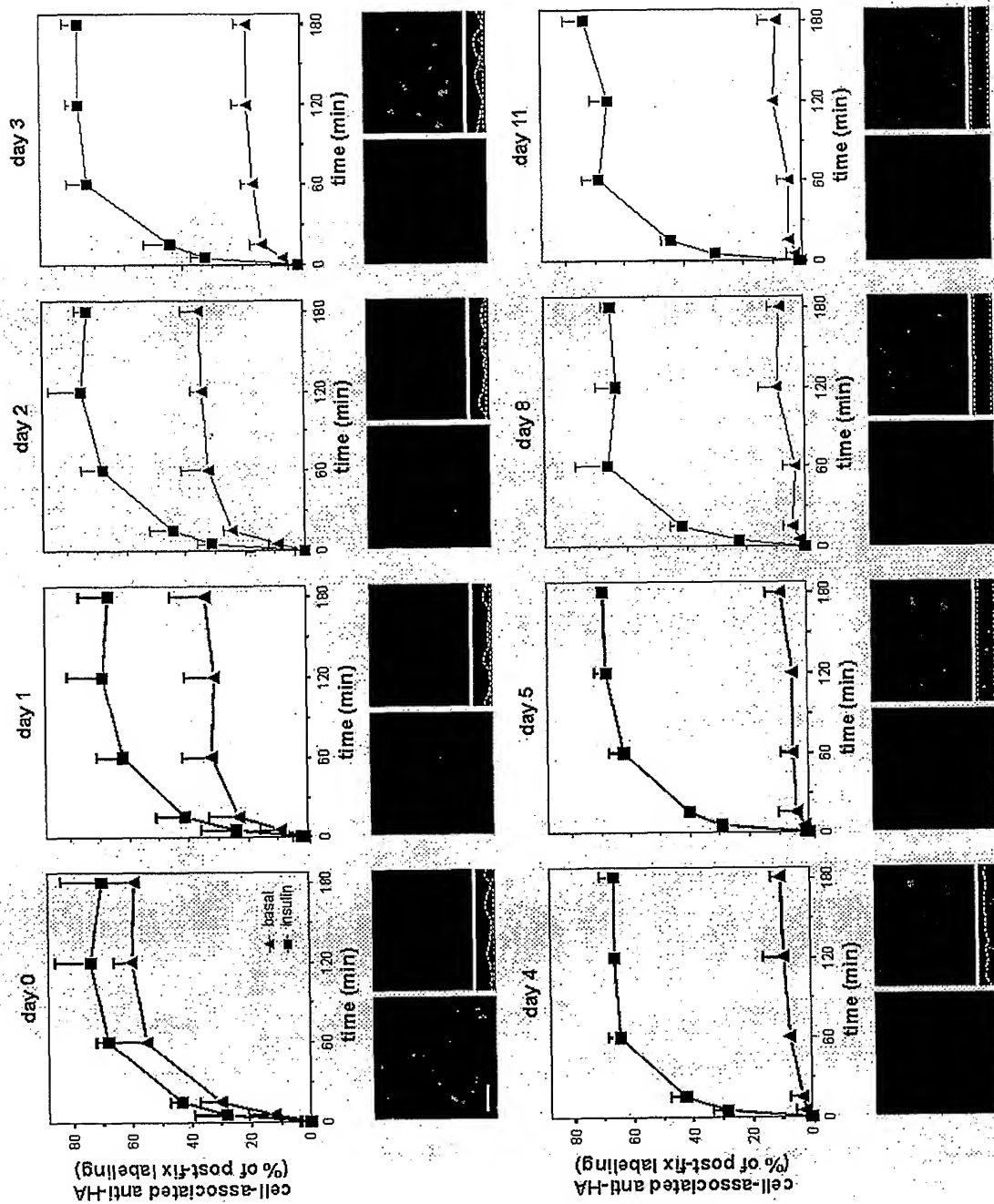


Figure 7

29/37

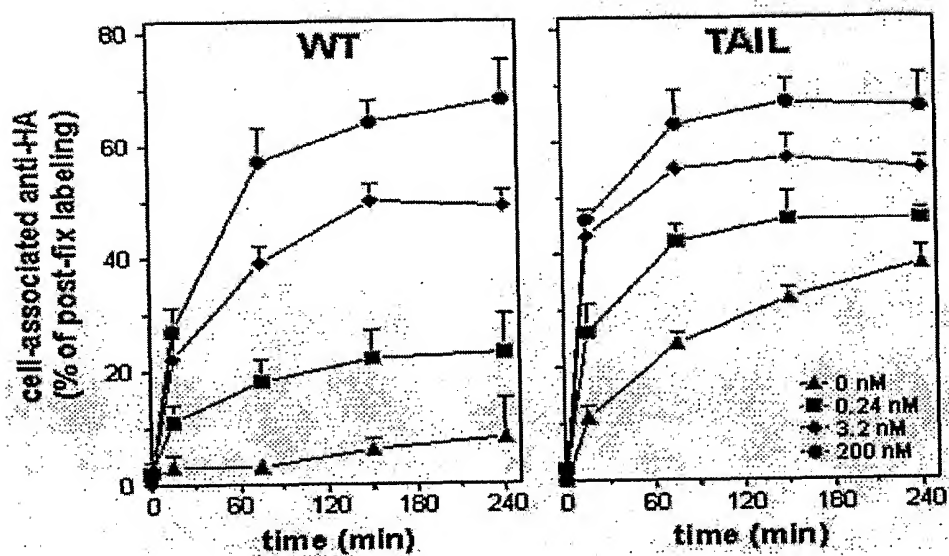


Figure 8a

30/37

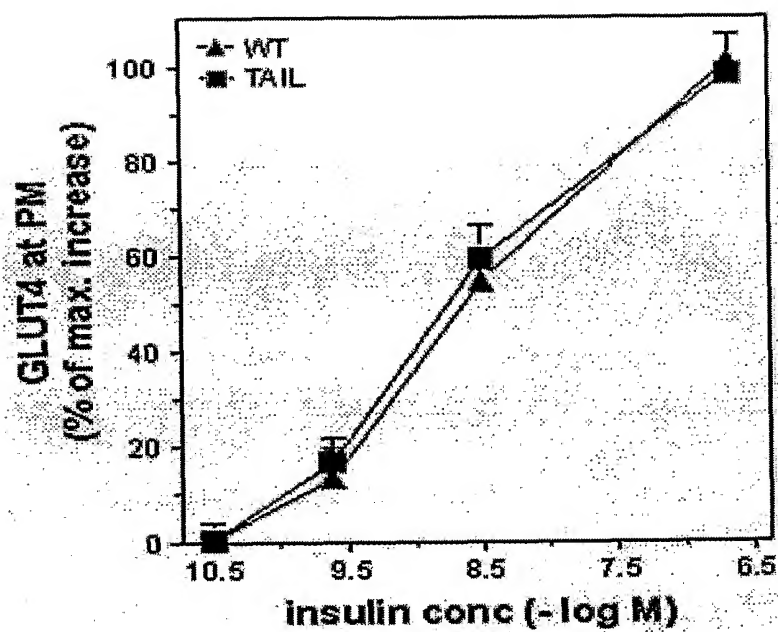


Figure 8b

31/37

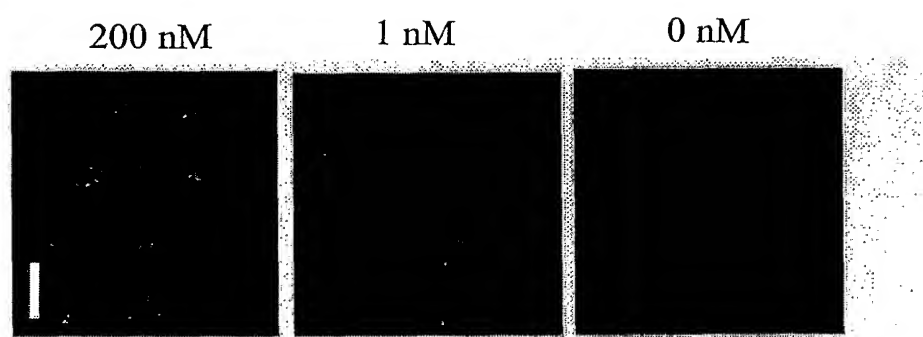


Figure 8c

32/37

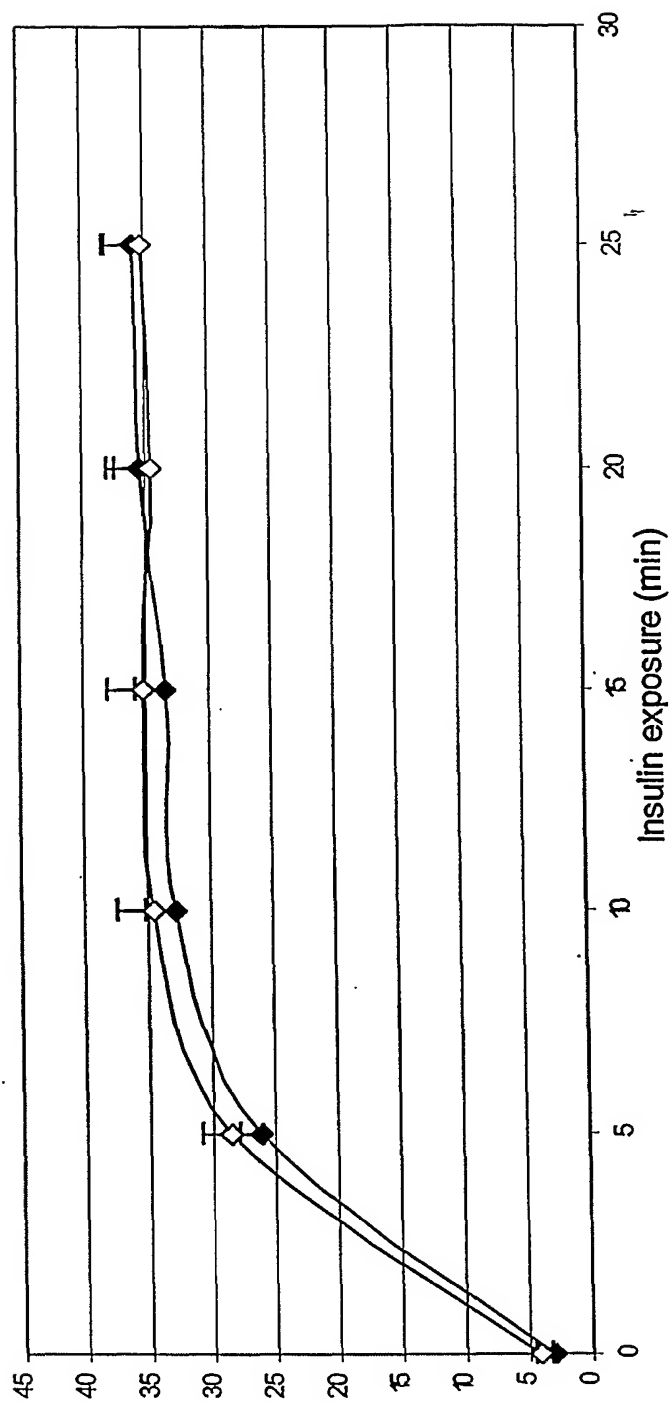


Figure 9

33/37

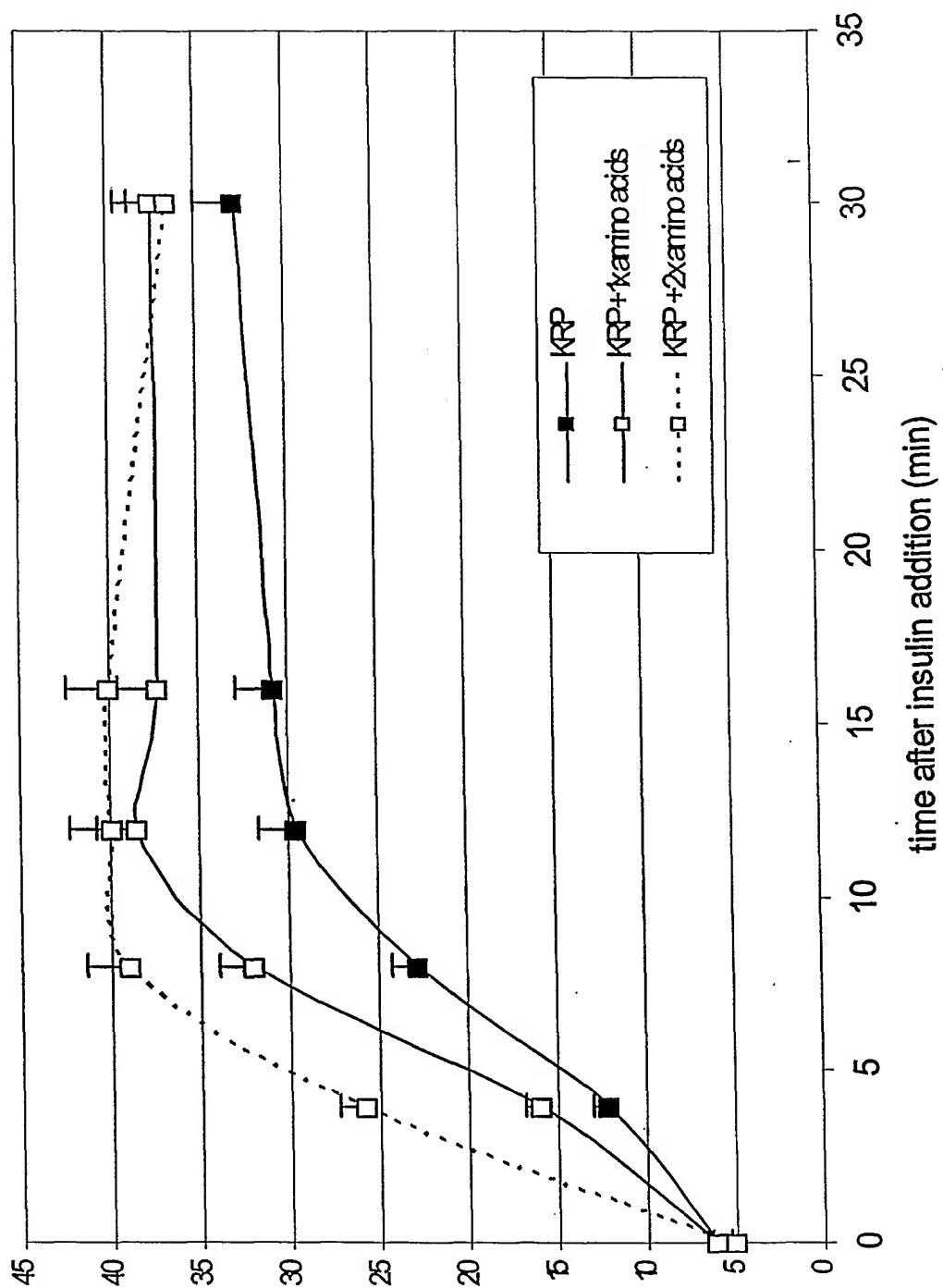


Figure 10

34/37

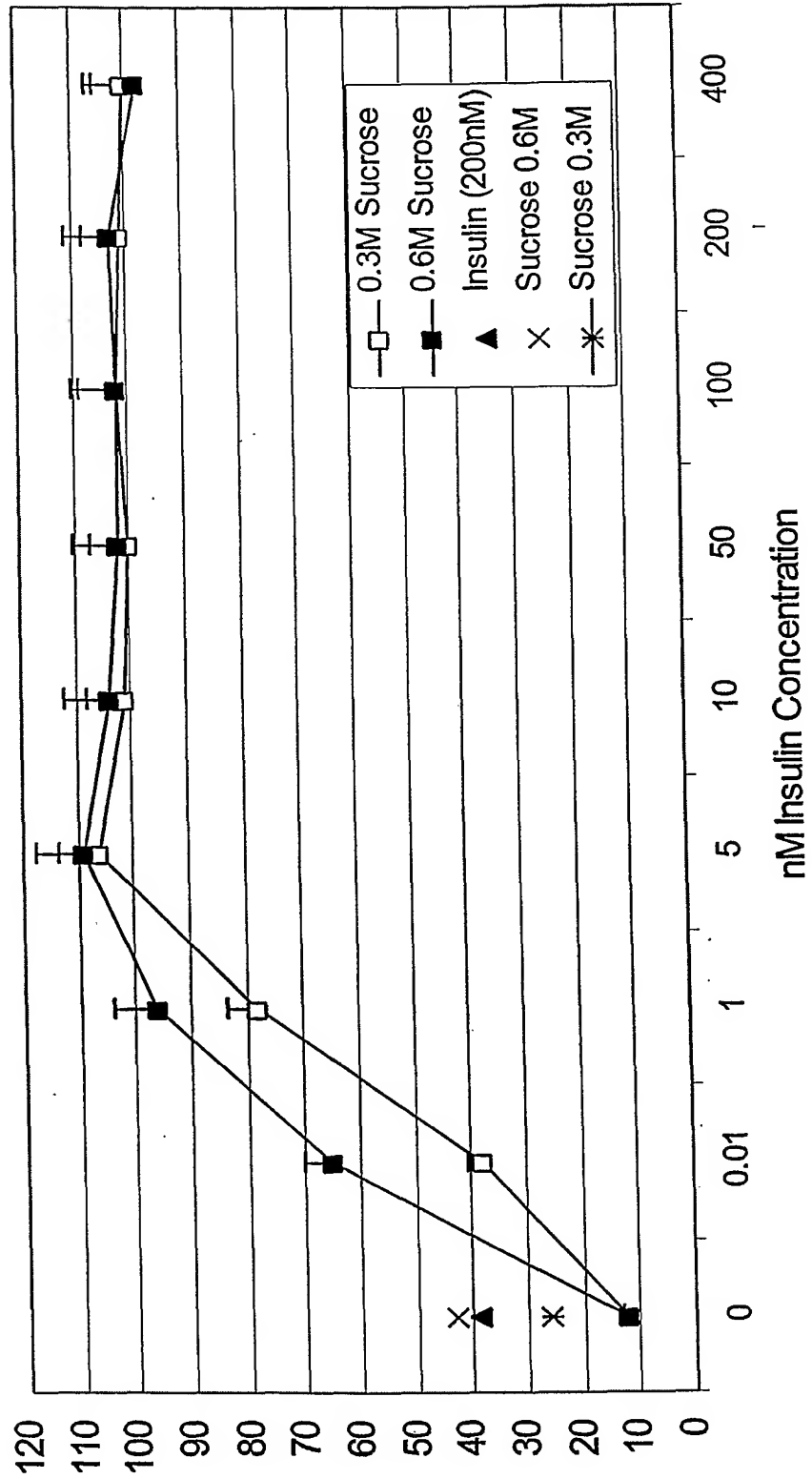


Figure 11

35/37



Figure 12A

36/37

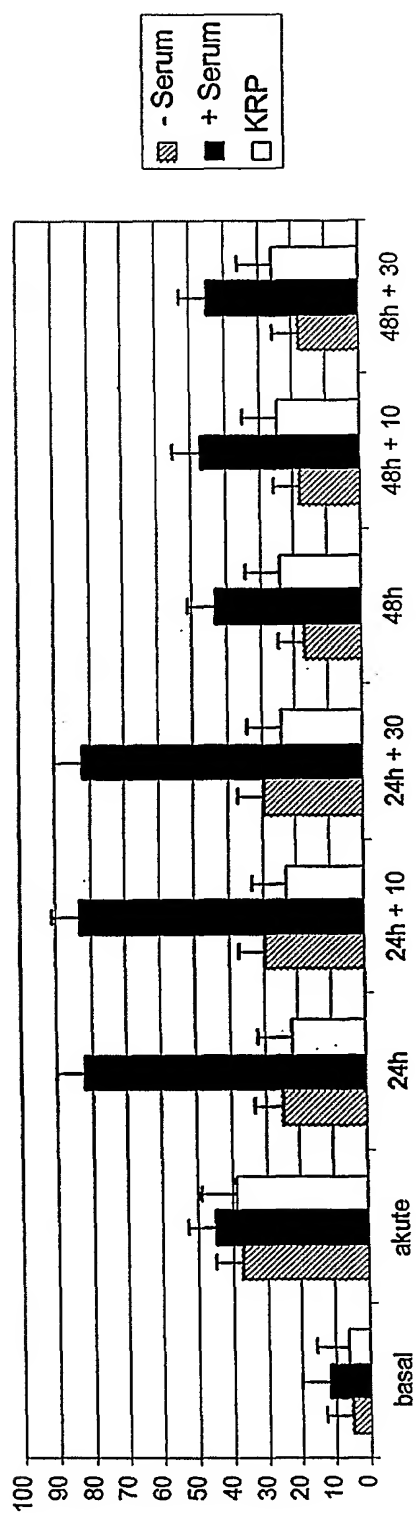


Figure 12B

37/37

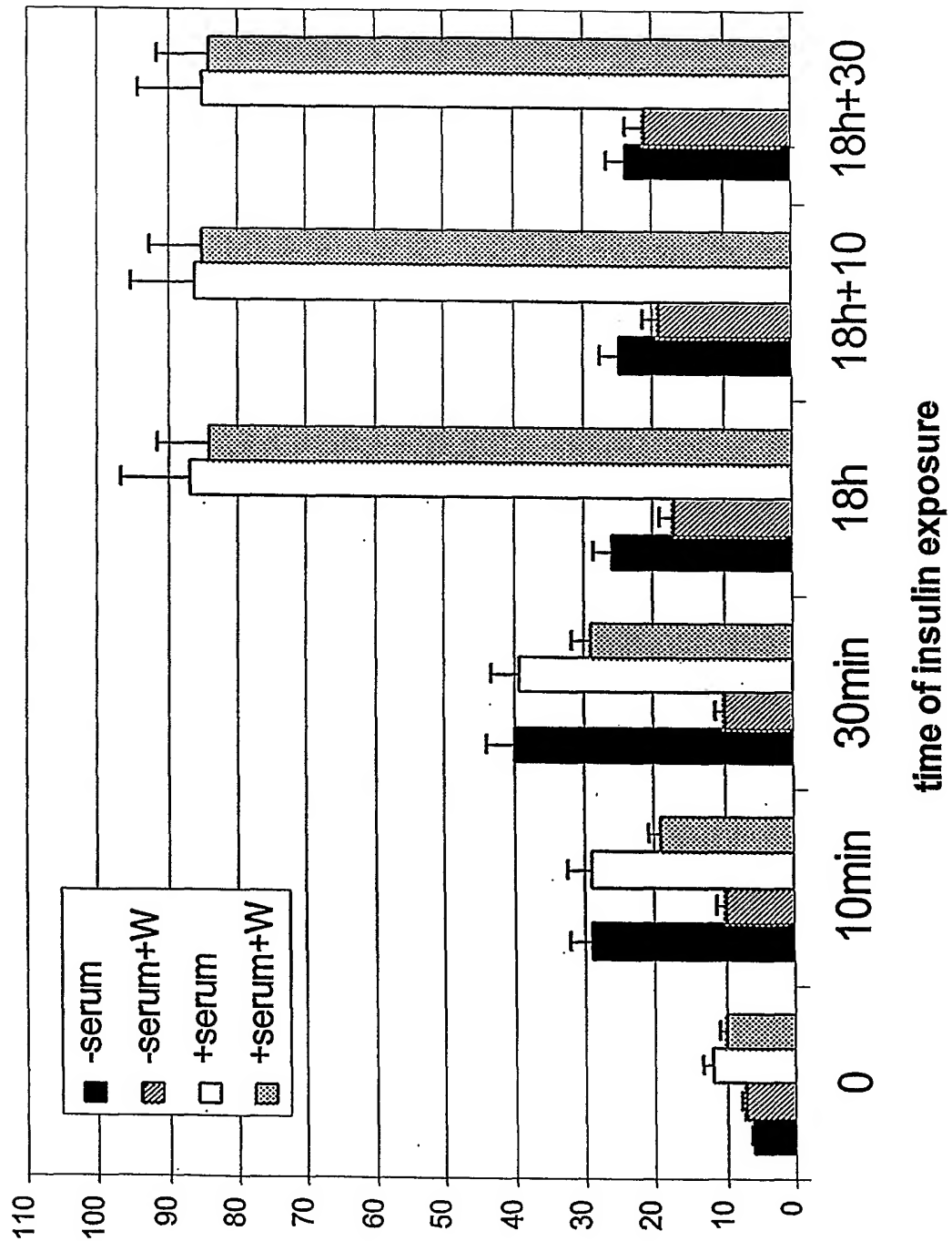


Figure 13

1

SEQUENCE LISTING

<110> Garvan Institute of Medical Research

<120> Novel Translocation Assay

<130> 502551/PXM

<150> AU2003904237

<151> 2003-08-08

<160> 64

<170> PatentIn version 3.1

<210> 1

<211> 2128

<212> DNA

<213> GLUT4

<220>

<221> CDS

<222> (146)..(1672)

<223>

<400> 1

```

gggggtccca tcgggccgc cctcgacgt cactccggga ccccgcggc ctccgcaggt      60
tctgcgtcc aggccggagt cagagactcc aggatcggtt ctttcatctt cgccgcccct      120
gcgcgtccag ctcttctaag acgag atg ccg tcg ggc ttc caa cag ata ggc      172
                               Met Pro Ser Gly Phe Gln Gln Ile Gly
                               1                               5

tcc gaa gat ggg gaa ccc cct cag cag cga gtg act ggg acc ctg gtc      220
Ser Glu Asp Gly Glu Pro Pro Gln Gln Arg Val Thr Gly Thr Leu Val
10                               15                               20                               25

ctt gct gtg ttc tct gcg gtg ctt ggc tcc ctg cag ttt ggg tac aac      268
Leu Ala Val Phe Ser Ala Val Leu Gly Ser Leu Gln Phe Gly Tyr Asn
                               30                               35                               40

att ggg gtc atc aat gcc cct cag aag gtg att gaa cag agc tac aat      316
Ile Gly Val Ile Asn Ala Pro Gln Lys Val Ile Glu Gln Ser Tyr Asn
                               45                               50                               55

gag acg tgg ctg ggg agg cag ggg cct gag gga ccc agc tcc atc cct      364
Glu Thr Trp Leu Gly Arg Gln Gly Pro Glu Gly Pro Ser Ser Ile Pro
60                               65                               70

cca ggc acc ctc acc acc ctc tgg gcc ctc tcc gtg gcc atc ttt tcc      412
Pro Gly Thr Leu Thr Thr Leu Trp Ala Leu Ser Val Ala Ile Phe Ser
75                               80                               85

gtg ggc ggc atg att tcc tcc ttc ctc att ggt atc atc tct cag tgg      460
Val Gly Gly Met Ile Ser Ser Phe Leu Ile Gly Ile Ile Ser Gln Trp
90                               95                               100                               105

ctt gga agg aaa agg gcc atg ctg gtc aac aat gtc ctg gcg gtg ctg      508
Leu Gly Arg Lys Arg Ala Met Leu Val Asn Asn Val Leu Ala Val Leu
110                               115                               120

ggg ggc agc ctc atg ggc ctg gcc aac gct gct gcc tcc tat gaa atg      556

```

Gly	Gly	Ser	Leu	Met	Gly	Leu	Ala	Asn	Ala	Ala	Ala	Ser	Tyr	Glu	Met	
			125					130						135		
ctc	atc	ctt	gga	cga	ttc	ctc	att	ggc	gcc	tac	tca	ggg	ctg	aca	tca	604
Leu	Ile	Leu	Gly	Arg	Phe	Leu	Ile	Gly	Ala	Tyr	Ser	Gly	Leu	Thr	Ser	
		140					145					150				
ggg	ctg	gtg	ccc	atg	tac	gtg	ggg	gag	att	gct	ccc	act	cac	ctg	cgg	652
Gly	Leu	Val	Pro	Met	Tyr	Val	Gly	Glu	Ile	Ala	Pro	Thr	His	Leu	Arg	
	155					160					165					
ggc	gcc	ctg	ggg	acg	ctc	aac	caa	ctg	gcc	att	gtt	atc	ggc	att	ctg	700
Gly	Ala	Leu	Gly	Thr	Leu	Asn	Gln	Leu	Ala	Ile	Val	Ile	Gly	Ile	Leu	
170					175					180					185	
atc	gcc	cag	gtg	ctg	ggc	ttg	gag	tcc	ctc	ctg	ggc	act	gcc	agc	ctg	748
Ile	Ala	Gln	Val	Leu	Gly	Leu	Glu	Ser	Leu	Leu	Gly	Thr	Ala	Ser	Leu	
				190					195					200		
tgg	cca	ctg	ctc	ctg	ggc	ctc	aca	gtg	cta	cct	gcc	ctc	ctg	cag	ctg	796
Trp	Pro	Leu	Leu	Leu	Gly	Leu	Thr	Val	Leu	Pro	Ala	Leu	Leu	Gln	Leu	
		205						210					215			
gtc	ctg	ctg	ccc	ttc	tgt	ccc	gag	agc	ccc	cgc	tac	ctc	tac	atc	atc	844
Val	Leu	Leu	Pro	Phe	Cys	Pro	Glu	Ser	Pro	Arg	Tyr	Leu	Tyr	Ile	Ile	
		220					225					230				
cag	aat	ctc	gag	ggg	cct	gcc	aga	aag	agt	ctg	aag	cgc	ctg	aca	ggc	892
Gln	Asn	Leu	Glu	Gly	Pro	Ala	Arg	Lys	Ser	Leu	Lys	Arg	Leu	Thr	Gly	
	235					240					245					
tgg	gcc	gat	gtt	tct	gga	gtg	ctg	gct	gag	ctg	aag	gat	gag	aag	cgg	940
Trp	Ala	Asp	Val	Ser	Gly	Val	Leu	Ala	Glu	Leu	Lys	Asp	Glu	Lys	Arg	
250					255					260					265	
aag	ctg	gag	cgt	gag	cgg	cca	ctg	tcc	ctg	ctc	cag	ctc	ctg	ggc	agc	988
Lys	Leu	Glu	Arg	Glu	Arg	Pro	Leu	Ser	Leu	Leu	Gln	Leu	Leu	Gly	Ser	
			270					275						280		
cgt	acc	cac	cgg	cag	ccc	ctg	atc	att	gcg	gtc	gtg	ctg	cag	ctg	agc	1036
Arg	Thr	His	Arg	Gln	Pro	Leu	Ile	Ile	Ala	Val	Val	Leu	Gln	Leu	Ser	
			285				290						295			
cag	cag	ctc	tct	ggc	atc	aat	gct	gtt	ttc	tat	tat	tcg	acc	agc	atc	1084
Gln	Gln	Leu	Ser	Gly	Ile	Asn	Ala	Val	Phe	Tyr	Tyr	Ser	Thr	Ser	Ile	
		300					305					310				
ttc	gag	aca	gca	ggg	gta	ggc	cag	cct	gcc	tat	gcc	acc	ata	gga	gct	1132
Phe	Glu	Thr	Ala	Gly	Val	Gly	Gln	Pro	Ala	Tyr	Ala	Thr	Ile	Gly	Ala	
	315					320					325					
ggt	gtg	gtc	aac	aca	gtc	ttc	acc	ttg	gtc	tcg	gtg	ttg	ttg	gtg	gag	1180
Gly	Val	Val	Asn	Thr	Val	Phe	Thr	Leu	Val	Ser	Val	Leu	Leu	Val	Glu	
330					335					340					345	
cgg	gcg	ggg	cgc	cgg	acg	ctc	cat	ctc	ctg	ggc	ctg	gcg	ggc	atg	tgt	1228
Arg	Ala	Gly	Arg	Arg	Thr	Leu	His	Leu	Leu	Gly	Leu	Ala	Gly	Met	Cys	
			350						355					360		
ggc	tgt	gcc	atc	ctg	atg	act	gtg	gct	ctg	ctc	ctg	ctg	gag	cga	gtt	1276
Gly	Cys	Ala	Ile	Leu	Met	Thr	Val	Ala	Leu	Leu	Leu	Leu	Glu	Arg	Val	
			365					370					375			

cca gcc atg agc tac gtc tcc att gtg gcc atc ttt ggc ttc gtg gca 1324
 Pro Ala Met Ser Tyr Val Ser Ile Val Ala Ile Phe Gly Phe Val Ala
 380 385 390

ttt ttt gag att ggc cct ggc ccc att cct tgg ttc atc gtg gcc gag 1372
 Phe Phe Glu Ile Gly Pro Gly Pro Ile Pro Trp Phe Ile Val Ala Glu
 395 400 405

ctc ttc agc cag gga ccc cgc ccg gca gcc atg gct gtg gct ggt ttc 1420
 Leu Phe Ser Gln Gly Pro Arg Pro Ala Ala Met Ala Val Ala Gly Phe
 410 415 420 425

tcc aac tgg acg agc aac ttc atc att ggc atg ggt ttc cag tat gtt 1468
 Ser Asn Trp Thr Ser Asn Phe Ile Ile Gly Met Gly Phe Gln Tyr Val
 430 435 440

gcg gag gct atg ggg ccc tac gtc ttc ctt cta ttt gcg gtc ctc ctg 1516
 Ala Glu Ala Met Gly Pro Tyr Val Phe Leu Leu Phe Ala Val Leu Leu
 445 450 455

ctg ggc ttc ttc atc ttc acc ttc tta aga gta cct gaa act cga ggc 1564
 Leu Gly Phe Phe Ile Phe Thr Phe Leu Arg Val Pro Glu Thr Arg Gly
 460 465 470

cgg acg ttt gac cag atc tca gct gcc ttc cac cgg aca ccc tct ctt 1612
 Arg Thr Phe Asp Gln Ile Ser Ala Ala Phe His Arg Thr Pro Ser Leu
 475 480 485

tta gag cag gag gtg aaa ccc agc aca gaa ctt gag tat tta ggg cca 1660
 Leu Glu Gln Glu Val Lys Pro Ser Thr Glu Leu Glu Tyr Leu Gly Pro
 490 495 500 505

gat gag aac gac tgaggggcca ggcaggggtg ggagagccag ctctctctac 1712
 Asp Glu Asn Asp

ccggccccaga gaccccttcc tttcctctgc agcactttaa ccctctcttc cctattattt 1772

ccgggtggaa aagaatccct gcagcctggt agaattggga agctggggga aggggtggtct 1832

gagcaccccc tcattcccct cgtgtgactc tcttgatta tttatgtgtt gtggtttggc 1892

cgtggccatc aggggtgggcc actctcccct cctcttccct tccccatcc cctttcctcc 1952

ccaccttccc cagactcagc tccagaatac cttcttcgct gctagagaag ggggattgga 2012

gggaagacag gtctagactt tctcagtggg acaaaccaga gcagagagca ggacaggaga 2072

caagaaatcc agtttccac caccttgac tctccaca atctgggact ttcact 2128

<210> 2
 <211> 509
 <212> PRT
 <213> GLUT4

<400> 2

Met Pro Ser Gly Phe Gln Gln Ile Gly Ser Glu Asp Gly Glu Pro Pro
 1 5 10 15

Gln Gln Arg Val Thr Gly Thr Leu Val Leu Ala Val Phe Ser Ala Val

			20						25					30			
Leu	Gly	Ser 35	Leu	Gln	Phe	Gly	Tyr 40	Asn	Ile	Gly	Val	Ile 45	Asn	Ala	Pro		
Gln	Lys 50	Val	Ile	Glu	Gln	Ser 55	Tyr	Asn	Glu	Thr	Trp 60	Leu	Gly	Arg	Gln		
Gly 65	Pro	Glu	Gly	Pro	Ser 70	Ser	Ile	Pro	Pro	Gly 75	Thr	Leu	Thr	Thr	Leu 80		
Trp	Ala	Leu	Ser	Val 85	Ala	Ile	Phe	Ser	Val 90	Gly	Gly	Met	Ile	Ser 95	Ser		
Phe	Leu	Ile	Gly 100	Ile	Ile	Ser	Gln	Trp 105	Leu	Gly	Arg	Lys	Arg 110	Ala	Met		
Leu	Val	Asn 115	Asn	Val	Leu	Ala	Val 120	Leu	Gly	Gly	Ser	Leu 125	Met	Gly	Leu		
Ala	Asn 130	Ala	Ala	Ala	Ser	Tyr 135	Glu	Met	Leu	Ile	Leu 140	Gly	Arg	Phe	Leu		
Ile 145	Gly	Ala	Tyr	Ser	Gly 150	Leu	Thr	Ser	Gly	Leu 155	Val	Pro	Met	Tyr	Val 160		
Gly	Glu	Ile	Ala	Pro 165	Thr	His	Leu	Arg	Gly 170	Ala	Leu	Gly	Thr	Leu 175	Asn		
Gln	Leu	Ala	Ile 180	Val	Ile	Gly	Ile 185	Leu	Ile	Ala	Gln	Val	Leu 190	Gly	Leu		
Glu	Ser 195	Leu	Leu	Gly	Thr	Ala	Ser 200	Leu	Trp	Pro	Leu	Leu 205	Leu	Gly	Leu		
Thr 210	Val	Leu	Pro	Ala	Leu	Leu 215	Gln	Leu	Val	Leu	Leu 220	Pro	Phe	Cys	Pro		
Glu 225	Ser	Pro	Arg	Tyr	Leu 230	Tyr	Ile	Ile	Gln	Asn 235	Leu	Glu	Gly	Pro	Ala 240		
Arg	Lys	Ser	Leu	Lys 245	Arg	Leu	Thr	Gly	Trp 250	Ala	Asp	Val	Ser	Gly 255	Val		
Leu	Ala	Glu	Leu 260	Lys	Asp	Glu	Lys	Arg 265	Lys	Leu	Glu	Arg	Glu 270	Arg	Pro		

Leu Ser Leu Leu Gln Leu Leu Gly Ser Arg Thr His Arg Gln Pro Leu
 275 280 285

Ile Ile Ala Val Val Leu Gln Leu Ser Gln Gln Leu Ser Gly Ile Asn
 290 295 300

Ala Val Phe Tyr Tyr Ser Thr Ser Ile Phe Glu Thr Ala Gly Val Gly
 305 310 315 320

Gln Pro Ala Tyr Ala Thr Ile Gly Ala Gly Val Val Asn Thr Val Phe
 325 330 335

Thr Leu Val Ser Val Leu Leu Val Glu Arg Ala Gly Arg Arg Thr Leu
 340 345 350

His Leu Leu Gly Leu Ala Gly Met Cys Gly Cys Ala Ile Leu Met Thr
 355 360 365

Val Ala Leu Leu Leu Leu Glu Arg Val Pro Ala Met Ser Tyr Val Ser
 370 375 380

Ile Val Ala Ile Phe Gly Phe Val Ala Phe Phe Glu Ile Gly Pro Gly
 385 390 395 400

Pro Ile Pro Trp Phe Ile Val Ala Glu Leu Phe Ser Gln Gly Pro Arg
 405 410 415

Pro Ala Ala Met Ala Val Ala Gly Phe Ser Asn Trp Thr Ser Asn Phe
 420 425 430

Ile Ile Gly Met Gly Phe Gln Tyr Val Ala Glu Ala Met Gly Pro Tyr
 435 440 445

Val Phe Leu Leu Phe Ala Val Leu Leu Leu Gly Phe Phe Ile Phe Thr
 450 455 460

Phe Leu Arg Val Pro Glu Thr Arg Gly Arg Thr Phe Asp Gln Ile Ser
 465 470 475 480

Ala Ala Phe His Arg Thr Pro Ser Leu Leu Glu Gln Glu Val Lys Pro
 485 490 495

Ser Thr Glu Leu Glu Tyr Leu Gly Pro Asp Glu Asn Asp
 500 505

<210> 3
 <211> 1566
 <212> DNA

<213> HA tagged GLUT4

<220>

<221> CDS

<222> (1)..(1566)

<223>

<400> 3

atg ccg tcg ggc ttc caa cag ata ggc tcc gaa gat ggg gaa ccc cct	48
Met Pro Ser Gly Phe Gln Gln Ile Gly Ser Glu Asp Gly Glu Pro Pro	
1 5 10 15	
cag cag cga gtg act ggg acc ctg gtc ctt gct gtg ttc tct gcg gtg	96
Gln Gln Arg Val Thr Gly Thr Leu Val Leu Ala Val Phe Ser Ala Val	
20 25 30	
ctt ggc tcc ctg cag ttt ggg tac aac att ggg gtc atc aat gcc cct	144
Leu Gly Ser Leu Gln Phe Gly Tyr Asn Ile Gly Val Ile Asn Ala Pro	
35 40 45	
cag aag gtg att gaa cag agc tac aat gag acg tgg ctg ggg agg cag	192
Gln Lys Val Ile Glu Gln Ser Tyr Asn Glu Thr Trp Leu Gly Arg Gln	
50 55 60	
ggg cct gag atc gat tat cct tat gat gtt cct gat tat gct gag gga	240
Gly Pro Glu Ile Asp Tyr Pro Tyr Asp Val Pro Asp Tyr Ala Glu Gly	
65 70 75 80	
ccc agc tcc atc cct cca ggc acc ctc acc acc ctc tgg gcc ctc tcc	288
Pro Ser Ser Ile Pro Pro Gly Thr Leu Thr Thr Leu Trp Ala Leu Ser	
85 90 95	
gtg gcc atc ttt tcc gtg ggc ggc atg att tcc tcc ttc ctc att ggt	336
Val Ala Ile Phe Ser Val Gly Gly Met Ile Ser Ser Phe Leu Ile Gly	
100 105 110	
atc atc tct cag tgg ctt gga agg aaa agg gcc atg ctg gtc aac aat	384
Ile Ile Ser Gln Trp Leu Gly Arg Lys Arg Ala Met Leu Val Asn Asn	
115 120 125	
gtc ctg gcg gtg ctg ggg ggc agc ctc atg ggc ctg gcc aac gct gct	432
Val Leu Ala Val Leu Gly Gly Ser Leu Met Gly Leu Ala Asn Ala Ala	
130 135 140	
gcc tcc tat gaa atg ctc atc ctt gga cga ttc ctc att ggc gcc tac	480
Ala Ser Tyr Glu Met Leu Ile Leu Gly Arg Phe Leu Ile Gly Ala Tyr	
145 150 155 160	
tca ggg ctg aca tca ggg ctg gtg ccc atg tac gtg ggg gag att gct	528
Ser Gly Leu Thr Ser Gly Leu Val Pro Met Tyr Val Gly Glu Ile Ala	
165 170 175	
ccc act cac ctg cgg ggc gcc ctg ggg acg ctc aac caa ctg gcc att	576
Pro Thr His Leu Arg Gly Ala Leu Gly Thr Leu Asn Gln Leu Ala Ile	
180 185 190	
gtt atc ggc att ctg atc gcc cag gtg ctg ggc ttg gag tcc ctc ctg	624
Val Ile Gly Ile Leu Ile Ala Gln Val Leu Gly Leu Glu Ser Leu Leu	
195 200 205	
ggc act gcc agc ctg tgg cca ctg ctc ctg ggc ctc aca gtg cta cct	672
Gly Thr Ala Ser Leu Trp Pro Leu Leu Leu Gly Leu Thr Val Leu Pro	
210 215 220	

gcc ctc ctg cag ctg gtc ctg ctg ccc ttc tgt ccc gag agc ccc cgc Ala Leu Leu Gln Leu Val Leu Leu Pro Phe Cys Pro Glu Ser Pro Arg 225 230 235 240	720
tac ctc tac atc atc cag aat ctc gag ggg cct gcc aga aag agt ctg Tyr Leu Tyr Ile Ile Gln Asn Leu Glu Gly Pro Ala Arg Lys Ser Leu 245 250 255	768
aag cgc ctg aca ggc tgg gcc gat gtt tct gga gtg ctg gct gag ctg Lys Arg Leu Thr Gly Trp Ala Asp Val Ser Gly Val Leu Ala Glu Leu 260 265 270	816
aag gat gag aag cgg aag ctg gag cgt gag cgg cca ctg tcc ctg ctc Lys Asp Glu Lys Arg Lys Leu Glu Arg Glu Arg Pro Leu Ser Leu Leu 275 280 285	864
cag ctc ctg ggc agc cgt acc cac cgg cag ccc ctg atc att gcg gtc Gln Leu Leu Gly Ser Arg Thr His Arg Gln Pro Leu Ile Ile Ala Val 290 295 300	912
gtg ctg cag ctg agc cag cag ctc tct ggc atc aat gct gtt ttc tat Val Leu Gln Leu Ser Gln Gln Leu Ser Gly Ile Asn Ala Val Phe Tyr 305 310 315 320	960
tat tcg acc agc atc ttc gag aca gca ggg gta ggc cag cct gcc tat Tyr Ser Thr Ser Ile Phe Glu Thr Ala Gly Val Gly Gln Pro Ala Tyr 325 330 335	1008
gcc acc ata gga gct ggt gtg gtc aac aca gtc ttc acc ttg gtc tcg Ala Thr Ile Gly Ala Gly Val Val Asn Thr Val Phe Thr Leu Val Ser 340 345 350	1056
gtg ttg ttg gtg gag cgg gcg ggg cgc cgg acg ctc cat ctc ctg ggc Val Leu Leu Val Glu Arg Ala Gly Arg Arg Thr Leu His Leu Leu Gly 355 360 365	1104
ctg gcg ggc atg tgt ggc tgt gcc atc ctg atg act gtg gct ctg ctc Leu Ala Gly Met Cys Gly Cys Ala Ile Leu Met Thr Val Ala Leu Leu 370 375 380	1152
ctg ctg gag cga gtt cca gcc atg agc tac gtc tcc att gtg gcc atc Leu Leu Glu Arg Val Pro Ala Met Ser Tyr Val Ser Ile Val Ala Ile 385 390 395 400	1200
ttt ggc ttc gtg gca ttt ttt gag att ggc cct ggc ccc att cct tgg Phe Gly Phe Val Ala Phe Phe Glu Ile Gly Pro Gly Pro Ile Pro Trp 405 410 415	1248
ttc atc gtg gcc gag ctc ttc agc cag gga ccc cgc ccg gca gcc atg Phe Ile Val Ala Glu Leu Phe Ser Gln Gly Pro Arg Pro Ala Ala Met 420 425 430	1296
gct gtg gct ggt ttc tcc aac tgg acg agc aac ttc atc att ggc atg Ala Val Ala Gly Phe Ser Asn Trp Thr Ser Asn Phe Ile Ile Gly Met 435 440 445	1344
ggt ttc cag tat gtt gcg gag gct atg ggg ccc tac gtc ttc ctt cta Gly Phe Gln Tyr Val Ala Glu Ala Met Gly Pro Tyr Val Phe Leu Leu 450 455 460	1392
ttt gcg gtc ctc ctg ctg ggc ttc ttc atc ttc acc ttc tta aga gta Phe Ala Val Leu Leu Leu Gly Phe Phe Ile Phe Thr Phe Leu Arg Val	1440

465 470 475 480

cct gaa act cga ggc cgg acg ttt gac cag atc tca gct gcc ttc cac 1488
 Pro Glu Thr Arg Gly Arg Thr Phe Asp Gln Ile Ser Ala Ala Phe His
 485 490 495

cgg aca ccc tct ctt tta gag cag gag gtg aaa ccc agc aca gaa ctt 1536
 Arg Thr Pro Ser Leu Leu Glu Gln Glu Val Lys Pro Ser Thr Glu Leu
 500 505 510

gag tat tta ggg cca gat gag aac gac tga 1566
 Glu Tyr Leu Gly Pro Asp Glu Asn Asp
 515 520

<210> 4
 <211> 521
 <212> PRT
 <213> HA tagged GLUT4

<400> 4

Met Pro Ser Gly Phe Gln Gln Ile Gly Ser Glu Asp Gly Glu Pro Pro
 1 5 10 15

Gln Gln Arg Val Thr Gly Thr Leu Val Leu Ala Val Phe Ser Ala Val
 20 25 30

Leu Gly Ser Leu Gln Phe Gly Tyr Asn Ile Gly Val Ile Asn Ala Pro
 35 40 45

Gln Lys Val Ile Glu Gln Ser Tyr Asn Glu Thr Trp Leu Gly Arg Gln
 50 55 60

Gly Pro Glu Ile Asp Tyr Pro Tyr Asp Val Pro Asp Tyr Ala Glu Gly
 65 70 75 80

Pro Ser Ser Ile Pro Pro Gly Thr Leu Thr Thr Leu Trp Ala Leu Ser
 85 90 95

Val Ala Ile Phe Ser Val Gly Gly Met Ile Ser Ser Phe Leu Ile Gly
 100 105 110

Ile Ile Ser Gln Trp Leu Gly Arg Lys Arg Ala Met Leu Val Asn Asn
 115 120 125

Val Leu Ala Val Leu Gly Gly Ser Leu Met Gly Leu Ala Asn Ala Ala
 130 135 140

Ala Ser Tyr Glu Met Leu Ile Leu Gly Arg Phe Leu Ile Gly Ala Tyr
 145 150 155 160

Ser Gly Leu Thr Ser Gly Leu Val Pro Met Tyr Val Gly Glu Ile Ala

	165		170		175
Pro Thr His	Leu Arg Gly Ala Leu Gly	Thr Leu Asn Gln Leu Ala Ile			
	180	185	190		
Val Ile Gly	Ile Leu Ile Ala Gln Val Leu Gly	Leu Glu Ser Leu Leu			
	195	200	205		
Gly Thr Ala	Ser Leu Trp Pro Leu Leu Leu Gly	Leu Thr Val Leu Pro			
	210	215	220		
Ala Leu Leu	Gln Leu Val Leu Leu Pro Phe Cys Pro Glu Ser Pro Arg				
	225	230	235	240	
Tyr Leu Tyr	Ile Ile Gln Asn Leu Glu Gly Pro Ala Arg Lys Ser Leu				
	245	250	255		
Lys Arg Leu	Thr Gly Trp Ala Asp Val Ser Gly Val Leu Ala Glu Leu				
	260	265	270		
Lys Asp Glu	Lys Arg Lys Leu Glu Arg Glu Arg Pro Leu Ser Leu Leu				
	275	280	285		
Gln Leu Leu	Gly Ser Arg Thr His Arg Gln Pro Leu Ile Ile Ala Val				
	290	295	300		
Val Leu Gln	Leu Ser Gln Gln Leu Ser Gly Ile Asn Ala Val Phe Tyr				
	305	310	315	320	
Tyr Ser Thr	Ser Ile Phe Glu Thr Ala Gly Val Gly Gln Pro Ala Tyr				
	325	330	335		
Ala Thr Ile	Gly Ala Gly Val Val Asn Thr Val Phe Thr Leu Val Ser				
	340	345	350		
Val Leu Leu	Val Glu Arg Ala Gly Arg Arg Thr Leu His Leu Leu Gly				
	355	360	365		
Leu Ala Gly	Met Cys Gly Cys Ala Ile Leu Met Thr Val Ala Leu Leu				
	370	375	380		
Leu Leu Glu	Arg Val Pro Ala Met Ser Tyr Val Ser Ile Val Ala Ile				
	385	390	395	400	
Phe Gly Phe	Val Ala Phe Phe Glu Ile Gly Pro Gly Pro Ile Pro Trp				
	405	410	415		

Phe Ile Val Ala Glu Leu Phe Ser Gln Gly Pro Arg Pro Ala Ala Met
 420 425 430

Ala Val Ala Gly Phe Ser Asn Trp Thr Ser Asn Phe Ile Ile Gly Met
 435 440 445

Gly Phe Gln Tyr Val Ala Glu Ala Met Gly Pro Tyr Val Phe Leu Leu
 450 455 460

Phe Ala Val Leu Leu Leu Gly Phe Phe Ile Phe Thr Phe Leu Arg Val
 465 470 475 480

Pro Glu Thr Arg Gly Arg Thr Phe Asp Gln Ile Ser Ala Ala Phe His
 485 490 495

Arg Thr Pro Ser Leu Leu Glu Gln Glu Val Lys Pro Ser Thr Glu Leu
 500 505 510

Glu Tyr Leu Gly Pro Asp Glu Asn Asp
 515 520

<210> 5
 <211> 512
 <212> PRT
 <213> GLUT4 TAIL mutant

<400> 5

Met Pro Ser Gly Phe Gln Gln Ile Gly Ser Glu Asp Gly Glu Pro Pro
 1 5 10 15

Gln Gln Arg Val Thr Gly Thr Leu Val Leu Ala Val Phe Ser Ala Val
 20 25 30

Leu Gly Ser Leu Gln Phe Gly Tyr Asn Ile Gly Val Ile Asn Ala Pro
 35 40 45

Gln Lys Val Ile Glu Gln Ser Tyr Asn Glu Thr Trp Leu Gly Arg Gln
 50 55 60

Gly Pro Glu Ile Asp Glu Gly Pro Ser Ser Ile Pro Pro Gly Thr Leu
 65 70 75 80

Thr Thr Leu Trp Ala Leu Ser Val Ala Ile Phe Ser Val Gly Gly Met
 85 90 95

Ile Ser Ser Phe Leu Ile Gly Ile Ile Ser Gln Trp Leu Gly Arg Lys
 100 105 110

Arg Ala Met Leu Val Asn Asn Val Leu Ala Val Leu Gly Gly Ser Leu
 115 120 125

Met Gly Leu Ala Asn Ala Ala Ala Ser Tyr Glu Met Leu Ile Leu Gly
 130 135 140

Arg Phe Leu Ile Gly Ala Tyr Ser Gly Leu Thr Ser Gly Leu Val Pro
 145 150 155 160

Met Tyr Val Gly Glu Ile Ala Pro Thr His Leu Arg Gly Ala Leu Gly
 165 170 175

Thr Leu Asn Gln Leu Ala Ile Val Ile Gly Ile Leu Ile Ala Gln Val
 180 185 190

Leu Gly Leu Glu Ser Leu Leu Gly Thr Ala Ser Leu Trp Pro Leu Leu
 195 200 205

Leu Gly Leu Thr Val Leu Pro Ala Leu Leu Gln Leu Val Leu Leu Pro
 210 215 220

Phe Cys Pro Glu Ser Pro Arg Tyr Leu Tyr Ile Ile Gln Asn Leu Glu
 225 230 235 240

Gly Pro Ala Arg Lys Ser Leu Lys Arg Leu Thr Gly Trp Ala Asp Val
 245 250 255

Ser Gly Val Leu Ala Glu Leu Lys Asp Glu Lys Arg Lys Leu Glu Arg
 260 265 270

Glu Arg Pro Leu Ser Leu Leu Gln Leu Leu Gly Ser Arg Thr His Arg
 275 280 285

Gln Pro Leu Ile Ile Ala Val Val Leu Gln Leu Ser Gln Gln Leu Ser
 290 295 300

Gly Ile Asn Ala Val Phe Tyr Tyr Ser Thr Ser Ile Phe Glu Thr Ala
 305 310 315 320

Gly Val Gly Gln Pro Ala Tyr Ala Thr Ile Gly Ala Gly Val Val Asn
 325 330 335

Thr Val Phe Thr Leu Val Ser Val Leu Leu Val Glu Arg Ala Gly Arg
 340 345 350

Arg Thr Leu His Leu Leu Gly Leu Ala Gly Met Cys Gly Cys Ala Ile
 355 360 365

12

Leu Met Thr Val Ala Leu Leu Leu Leu Glu Arg Val Pro Ala Met Ser
370 375 380

Tyr Val Ser Ile Val Ala Ile Phe Gly Phe Val Ala Phe Phe Glu Ile
385 390 395 400

Gly Pro Gly Pro Ile Pro Trp Phe Ile Val Ala Glu Leu Phe Ser Gln
405 410 415

Gly Pro Arg Pro Ala Ala Met Ala Val Ala Gly Phe Ser Asn Trp Thr
420 425 430

Ser Asn Phe Ile Ile Gly Met Gly Phe Gln Tyr Val Ala Glu Ala Met
435 440 445

Gly Pro Tyr Val Phe Leu Leu Phe Ala Val Leu Leu Leu Gly Phe Phe
450 455 460

Ile Phe Thr Phe Leu Arg Val Pro Glu Thr Arg Gly Arg Thr Phe Asp
465 470 475 480

Gln Ile Ser Ala Ala Phe His Arg Thr Pro Ser Leu Leu Glu Gln Glu
485 490 495

Val Lys Pro Ser Ser Ile Glu Pro Ala Lys Glu Thr Thr Thr Asn Val
500 505 510

<210> 6

<211> 512

<212> PRT

<213> HA tagged GLUT4 TAIL mutant

MPSGFQQIGSEDGEPPQQRVTGTLVLAVFSAVLGSLSQFGYNIGVINAPQKVIEQSYNETWLGRQGPEIDYPYDVPDYAE
GPSSIPPGLTTLWALSVAIFSVGGMISSFLIGIISQWLGRKRAMLVNNVLAVLGGSLMGLANAAASYEMLILGRFLIG
AYSGLTSGLVPMYVGEIAPTHLRGALGTNLQLAIVIGILIAQVLGLESLLGTASLWPLLLGLTVLPALLQLVLLPFCPE
SPRYLYIIQNLEGPARKSLKRLTGWADVSGVLAELKDEKRKLERERPLSLLQLLGSRTHRQPLIIAVVLQLSQQLSGIN
AVFYYSTSIFETAGVGQPAYATIGAGVNTVFTLVSVLLVERAGRRTLHLLGLAGMCGCAILMTVALLLLERVPAHSV
SIVAIFGFVAFFEIGPGPIPWFIIVAELEFSQGPRPAAMAVAGFSNWTSTNFIIGMGFYVAEAMGPYVFLLEFAVLLLGFFI
FTFLRVPETRGRTFDQISAAFHRTPLLEQEVKPSIEPAKETTTNV

<seq007>prt/1;GLUT4 L489,490A mutant

<400> 6

Met Pro Ser Gly Phe Gln Gln Ile Gly Ser Glu Asp Gly Glu Pro Pro
1 5 10 15

Gln Gln Arg Val Thr Gly Thr Leu Val Leu Ala Val Phe Ser Ala Val
20 25 30

Leu Gly Ser Leu Gln Phe Gly Tyr Asn Ile Gly Val Ile Asn Ala Pro
35 40 45

Gln Lys Val Ile Glu Gln Ser Tyr Asn Glu Thr Trp Leu Gly Arg Gln
 50 55 60

Gly Pro Glu Ile Asp Glu Gly Pro Ser Ser Ile Pro Pro Gly Thr Leu
 65 70 75 80

Thr Thr Leu Trp Ala Leu Ser Val Ala Ile Phe Ser Val Gly Gly Met
 85 90 95

Ile Ser Ser Phe Leu Ile Gly Ile Ile Ser Gln Trp Leu Gly Arg Lys
 100 105 110

Arg Ala Met Leu Val Asn Asn Val Leu Ala Val Leu Gly Gly Ser Leu
 115 120 125

Met Gly Leu Ala Asn Ala Ala Ala Ser Tyr Glu Met Leu Ile Leu Gly
 130 135 140

Arg Phe Leu Ile Gly Ala Tyr Ser Gly Leu Thr Ser Gly Leu Val Pro
 145 150 155 160

Met Tyr Val Gly Glu Ile Ala Pro Thr His Leu Arg Gly Ala Leu Gly
 165 170 175

Thr Leu Asn Gln Leu Ala Ile Val Ile Gly Ile Leu Ile Ala Gln Val
 180 185 190

Leu Gly Leu Glu Ser Leu Leu Gly Thr Ala Ser Leu Trp Pro Leu Leu
 195 200 205

Leu Gly Leu Thr Val Leu Pro Ala Leu Leu Gln Leu Val Leu Leu Pro
 210 215 220

Phe Cys Pro Glu Ser Pro Arg Tyr Leu Tyr Ile Ile Gln Asn Leu Glu
 225 230 235 240

Gly Pro Ala Arg Lys Ser Leu Lys Arg Leu Thr Gly Trp Ala Asp Val
 245 250 255

Ser Gly Val Leu Ala Glu Leu Lys Asp Glu Lys Arg Lys Leu Glu Arg
 260 265 270

Glu Arg Pro Leu Ser Leu Leu Gln Leu Leu Gly Ser Arg Thr His Arg
 275 280 285

Gln Pro Leu Ile Ile Ala Val Val Leu Gln Leu Ser Gln Gln Leu Ser
 290 295 300

14

Gly Ile Asn Ala Val Phe Tyr Tyr Ser Thr Ser Ile Phe Glu Thr Ala
305 310 315 320

Gly Val Gly Gln Pro Ala Tyr Ala Thr Ile Gly Ala Gly Val Val Asn
325 330 335

Thr Val Phe Thr Leu Val Ser Val Leu Leu Val Glu Arg Ala Gly Arg
340 345 350

Arg Thr Leu His Leu Leu Gly Leu Ala Gly Met Cys Gly Cys Ala Ile
355 360 365

Leu Met Thr Val Ala Leu Leu Leu Leu Glu Arg Val Pro Ala Met Ser
370 375 380

Tyr Val Ser Ile Val Ala Ile Phe Gly Phe Val Ala Phe Phe Glu Ile
385 390 395 400

Gly Pro Gly Pro Ile Pro Trp Phe Ile Val Ala Glu Leu Phe Ser Gln
405 410 415

Gly Pro Arg Pro Ala Ala Met Ala Val Ala Gly Phe Ser Asn Trp Thr
420 425 430

Ser Asn Phe Ile Ile Gly Met Gly Phe Gln Tyr Val Ala Glu Ala Met
435 440 445

Gly Pro Tyr Val Phe Leu Leu Phe Ala Val Leu Leu Leu Gly Phe Phe
450 455 460

Ile Phe Thr Phe Leu Arg Val Pro Glu Thr Arg Gly Arg Thr Phe Asp
465 470 475 480

Gln Ile Ser Ala Ala Phe His Arg Thr Pro Ser Ala Ala Glu Gln Glu
485 490 495

Val Lys Pro Ser Thr Glu Leu Glu Tyr Leu Gly Pro Asp Glu Asn Asp
500 505 510

<210> 7
<211> 521
<212> PRT
<213> HA tagged GLUT4 L489,490A mutant

<400> 7

Met Pro Ser Gly Phe Gln Gln Ile Gly Ser Glu Asp Gly Glu Pro Pro
1 5 10 15

Gln Gln Arg Val Thr Gly Thr Leu Val Leu Ala Val Phe Ser Ala Val
 20 25 30
 Leu Gly Ser Leu Gln Phe Gly Tyr Asn Ile Gly Val Ile Asn Ala Pro
 35 40 45
 Gln Lys Val Ile Glu Gln Ser Tyr Asn Glu Thr Trp Leu Gly Arg Gln
 50 55 60
 Gly Pro Glu Ile Asp Tyr Pro Tyr Asp Val Pro Asp Tyr Ala Glu Gly
 65 70 75 80
 Pro Ser Ser Ile Pro Pro Gly Thr Leu Thr Thr Leu Trp Ala Leu Ser
 85 90 95
 Val Ala Ile Phe Ser Val Gly Gly Met Ile Ser Ser Phe Leu Ile Gly
 100 105 110
 Ile Ile Ser Gln Trp Leu Gly Arg Lys Arg Ala Met Leu Val Asn Asn
 115 120 125
 Val Leu Ala Val Leu Gly Gly Ser Leu Met Gly Leu Ala Asn Ala Ala
 130 135 140
 Ala Ser Tyr Glu Met Leu Ile Leu Gly Arg Phe Leu Ile Gly Ala Tyr
 145 150 155 160
 Ser Gly Leu Thr Ser Gly Leu Val Pro Met Tyr Val Gly Glu Ile Ala
 165 170 175
 Pro Thr His Leu Arg Gly Ala Leu Gly Thr Leu Asn Gln Leu Ala Ile
 180 185 190
 Val Ile Gly Ile Leu Ile Ala Gln Val Leu Gly Leu Glu Ser Leu Leu
 195 200 205
 Gly Thr Ala Ser Leu Trp Pro Leu Leu Leu Gly Leu Thr Val Leu Pro
 210 215 220
 Ala Leu Leu Gln Leu Val Leu Leu Pro Phe Cys Pro Glu Ser Pro Arg
 225 230 235 240
 Tyr Leu Tyr Ile Ile Gln Asn Leu Glu Gly Pro Ala Arg Lys Ser Leu
 245 250 255
 Lys Arg Leu Thr Gly Trp Ala Asp Val Ser Gly Val Leu Ala Glu Leu

260	265	270
Lys Asp Glu Lys Arg Lys Leu Glu Arg Glu Arg Pro Leu Ser Leu Leu 275 280 285		
Gln Leu Leu Gly Ser Arg Thr His Arg Gln Pro Leu Ile Ile Ala Val 290 295 300		
Val Leu Gln Leu Ser Gln Gln Leu Ser Gly Ile Asn Ala Val Phe Tyr 305 310 315 320		
Tyr Ser Thr Ser Ile Phe Glu Thr Ala Gly Val Gly Gln Pro Ala Tyr 325 330 335		
Ala Thr Ile Gly Ala Gly Val Val Asn Thr Val Phe Thr Leu Val Ser 340 345 350		
Val Leu Leu Val Glu Arg Ala Gly Arg Arg Thr Leu His Leu Leu Gly 355 360 365		
Leu Ala Gly Met Cys Gly Cys Ala Ile Leu Met Thr Val Ala Leu Leu 370 375 380		
Leu Leu Glu Arg Val Pro Ala Met Ser Tyr Val Ser Ile Val Ala Ile 385 390 395 400		
Phe Gly Phe Val Ala Phe Phe Glu Ile Gly Pro Gly Pro Ile Pro Trp 405 410 415		
Phe Ile Val Ala Glu Leu Phe Ser Gln Gly Pro Arg Pro Ala Ala Met 420 425 430		
Ala Val Ala Gly Phe Ser Asn Trp Thr Ser Asn Phe Ile Ile Gly Met 435 440 445		
Gly Phe Gln Tyr Val Ala Glu Ala Met Gly Pro Tyr Val Phe Leu Leu 450 455 460		
Phe Ala Val Leu Leu Leu Gly Phe Phe Ile Phe Thr Phe Leu Arg Val 465 470 475 480		
Pro Glu Thr Arg Gly Arg Thr Phe Asp Gln Ile Ser Ala Ala Phe His 485 490 495		
Arg Thr Pro Ser Ala Ala Glu Gln Glu Val Lys Pro Ser Thr Glu Leu 500 505 510		

Glu Tyr Leu Gly Pro Asp Glu Asn Asp
 515 520

<210> 8
 <211> 512
 <212> PRT
 <213> GLUT4 F5A mutant

<400> 8

Met Pro Ser Gly Ala Gln Gln Ile Gly Ser Glu Asp Gly Glu Pro Pro
 1 5 10 15

Gln Gln Arg Val Thr Gly Thr Leu Val Leu Ala Val Phe Ser Ala Val
 20 25 30

Leu Gly Ser Leu Gln Phe Gly Tyr Asn Ile Gly Val Ile Asn Ala Pro
 35 40 45

Gln Lys Val Ile Glu Gln Ser Tyr Asn Glu Thr Trp Leu Gly Arg Gln
 50 55 60

Gly Pro Glu Ile Asp Glu Gly Pro Ser Ser Ile Pro Pro Gly Thr Leu
 65 70 75 80

Thr Thr Leu Trp Ala Leu Ser Val Ala Ile Phe Ser Val Gly Gly Met
 85 90 95

Ile Ser Ser Phe Leu Ile Gly Ile Ile Ser Gln Trp Leu Gly Arg Lys
 100 105 110

Arg Ala Met Leu Val Asn Asn Val Leu Ala Val Leu Gly Gly Ser Leu
 115 120 125

Met Gly Leu Ala Asn Ala Ala Ala Ser Tyr Glu Met Leu Ile Leu Gly
 130 135 140

Arg Phe Leu Ile Gly Ala Tyr Ser Gly Leu Thr Ser Gly Leu Val Pro
 145 150 155 160

Met Tyr Val Gly Glu Ile Ala Pro Thr His Leu Arg Gly Ala Leu Gly
 165 170 175

Thr Leu Asn Gln Leu Ala Ile Val Ile Gly Ile Leu Ile Ala Gln Val
 180 185 190

Leu Gly Leu Glu Ser Leu Leu Gly Thr Ala Ser Leu Trp Pro Leu Leu
 195 200 205

Leu Gly Leu Thr Val Leu Pro Ala Leu Leu Gln Leu Val Leu Leu Pro
 210 215 220

Phe Cys Pro Glu Ser Pro Arg Tyr Leu Tyr Ile Ile Gln Asn Leu Glu
 225 230 235 240

Gly Pro Ala Arg Lys Ser Leu Lys Arg Leu Thr Gly Trp Ala Asp Val
 245 250 255

Ser Gly Val Leu Ala Glu Leu Lys Asp Glu Lys Arg Lys Leu Glu Arg
 260 265 270

Glu Arg Pro Leu Ser Leu Leu Gln Leu Leu Gly Ser Arg Thr His Arg
 275 280 285

Gln Pro Leu Ile Ile Ala Val Val Leu Gln Leu Ser Gln Gln Leu Ser
 290 295 300

Gly Ile Asn Ala Val Phe Tyr Tyr Ser Thr Ser Ile Phe Glu Thr Ala
 305 310 315 320

Gly Val Gly Gln Pro Ala Tyr Ala Thr Ile Gly Ala Gly Val Val Asn
 325 330 335

Thr Val Phe Thr Leu Val Ser Val Leu Leu Val Glu Arg Ala Gly Arg
 340 345 350

Arg Thr Leu His Leu Leu Gly Leu Ala Gly Met Cys Gly Cys Ala Ile
 355 360 365

Leu Met Thr Val Ala Leu Leu Leu Leu Glu Arg Val Pro Ala Met Ser
 370 375 380

Tyr Val Ser Ile Val Ala Ile Phe Gly Phe Val Ala Phe Phe Glu Ile
 385 390 395 400

Gly Pro Gly Pro Ile Pro Trp Phe Ile Val Ala Glu Leu Phe Ser Gln
 405 410 415

Gly Pro Arg Pro Ala Ala Met Ala Val Ala Gly Phe Ser Asn Trp Thr
 420 425 430

Ser Asn Phe Ile Ile Gly Met Gly Phe Gln Tyr Val Ala Glu Ala Met
 435 440 445

Gly Pro Tyr Val Phe Leu Leu Phe Ala Val Leu Leu Leu Gly Phe Phe
 450 455 460

Ile Phe Thr Phe Leu Arg Val Pro Glu Thr Arg Gly Arg Thr Phe Asp
465 470 475 480

Gln Ile Ser Ala Ala Phe His Arg Thr Pro Ser Leu Leu Glu Gln Glu
485 490 495

Val Lys Pro Ser Thr Glu Leu Glu Tyr Leu Gly Pro Asp Glu Asn Asp
500 505 510

<210> 9
<211> 521
<212> PRT
<213> HA tagged GLUT4 F5A mutant

<400> 9

Met Pro Ser Gly Ala Gln Gln Ile Gly Ser Glu Asp Gly Glu Pro Pro
1 5 10 15

Gln Gln Arg Val Thr Gly Thr Leu Val Leu Ala Val Phe Ser Ala Val
20 25 30

Leu Gly Ser Leu Gln Phe Gly Tyr Asn Ile Gly Val Ile Asn Ala Pro
35 40 45

Gln Lys Val Ile Glu Gln Ser Tyr Asn Glu Thr Trp Leu Gly Arg Gln
50 55 60

Gly Pro Glu Ile Asp Tyr Pro Tyr Asp Val Pro Asp Tyr Ala Glu Gly
65 70 75 80

Pro Ser Ser Ile Pro Pro Gly Thr Leu Thr Thr Leu Trp Ala Leu Ser
85 90 95

Val Ala Ile Phe Ser Val Gly Gly Met Ile Ser Ser Phe Leu Ile Gly
100 105 110

Ile Ile Ser Gln Trp Leu Gly Arg Lys Arg Ala Met Leu Val Asn Asn
115 120 125

Val Leu Ala Val Leu Gly Gly Ser Leu Met Gly Leu Ala Asn Ala Ala
130 135 140

Ala Ser Tyr Glu Met Leu Ile Leu Gly Arg Phe Leu Ile Gly Ala Tyr
145 150 155 160

Ser Gly Leu Thr Ser Gly Leu Val Pro Met Tyr Val Gly Glu Ile Ala
165 170 175

Pro Thr His Leu Arg Gly Ala Leu Gly Thr Leu Asn Gln Leu Ala Ile
 180 185 190

Val Ile Gly Ile Leu Ile Ala Gln Val Leu Gly Leu Glu Ser Leu Leu
 195 200 205

Gly Thr Ala Ser Leu Trp Pro Leu Leu Leu Gly Leu Thr Val Leu Pro
 210 215 220

Ala Leu Leu Gln Leu Val Leu Leu Pro Phe Cys Pro Glu Ser Pro Arg
 225 230 235 240

Tyr Leu Tyr Ile Ile Gln Asn Leu Glu Gly Pro Ala Arg Lys Ser Leu
 245 250 255

Lys Arg Leu Thr Gly Trp Ala Asp Val Ser Gly Val Leu Ala Glu Leu
 260 265 270

Lys Asp Glu Lys Arg Lys Leu Glu Arg Glu Arg Pro Leu Ser Leu Leu
 275 280 285

Gln Leu Leu Gly Ser Arg Thr His Arg Gln Pro Leu Ile Ile Ala Val
 290 295 300

Val Leu Gln Leu Ser Gln Gln Leu Ser Gly Ile Asn Ala Val Phe Tyr
 305 310 315 320

Tyr Ser Thr Ser Ile Phe Glu Thr Ala Gly Val Gly Gln Pro Ala Tyr
 325 330 335

Ala Thr Ile Gly Ala Gly Val Val Asn Thr Val Phe Thr Leu Val Ser
 340 345 350

Val Leu Leu Val Glu Arg Ala Gly Arg Arg Thr Leu His Leu Leu Gly
 355 360 365

Leu Ala Gly Met Cys Gly Cys Ala Ile Leu Met Thr Val Ala Leu Leu
 370 375 380

Leu Leu Glu Arg Val Pro Ala Met Ser Tyr Val Ser Ile Val Ala Ile
 385 390 395 400

Phe Gly Phe Val Ala Phe Phe Glu Ile Gly Pro Gly Pro Ile Pro Trp
 405 410 415

Phe Ile Val Ala Glu Leu Phe Ser Gln Gly Pro Arg Pro Ala Ala Met
 420 425 430

Ala Val Ala Gly Phe Ser Asn Trp Thr Ser Asn Phe Ile Ile Gly Met
435 440 445

Gly Phe Gln Tyr Val Ala Glu Ala Met Gly Pro Tyr Val Phe Leu Leu
450 455 460

Phe Ala Val Leu Leu Leu Gly Phe Phe Ile Phe Thr Phe Leu Arg Val
465 470 475 480

Pro Glu Thr Arg Gly Arg Thr Phe Asp Gln Ile Ser Ala Ala Phe His
485 490 495

Arg Thr Pro Ser Leu Leu Glu Gln Glu Val Lys Pro Ser Thr Glu Leu
500 505 510

Glu Tyr Leu Gly Pro Asp Glu Asn Asp
515 520

<210> 10
<211> 2856
<212> DNA
<213> GLUT1

<220>
<221> CDS
<222> (180)..(1658)
<223>

<400> 10
tagtcgcgagg tccccgagtg agcacgccag ggagcaggag accaaacgac ggggggtcggg 60
gtcagagtcg cagtgggagt.ccccgaccg gagcacgagc ctgagcggga gagcgccgct 120
cgcacgcccg tcgccacccg cgtacccggc gcagccagag ccaccagcgc agcgctgcc 179
atg gag ccc agc agc aag aag ctg acg ggt cgc ctc atg ctg gct gtg 227
Met Glu Pro Ser Ser Lys Lys Leu Thr Gly Arg Leu Met Leu Ala Val
1 5 10 15
gga gga gca gtg ctt ggc tcc ctg cag ttt ggc tac aac act gga gtc 275
Gly Gly Ala Val Leu Gly Ser Leu Gln Phe Gly Tyr Asn Thr Gly Val
20 25 30
atc aat gcc ccc cag aag gtg atc gag gag ttc tac aac cag aca tgg 323
Ile Asn Ala Pro Gln Lys Val Ile Glu Glu Phe Tyr Asn Gln Thr Trp
35 40 45
gtc cac cgc tat ggg gag agc atc ctg ccc acc acg ctc acc acg ctc 371
Val His Arg Tyr Gly Glu Ser Ile Leu Pro Thr Thr Leu Thr Thr Leu
50 55 60
tgg tcc ctc tca gtg gcc atc ttt tct gtt ggg ggc atg att ggc tcc 419
Trp Ser Leu Ser Val Ala Ile Phe Ser Val Gly Gly Met Ile Gly Ser
65 70 75 80

ttc tct gtg ggc ctt ttc gtt aac cgc ttt ggc cgg cgg aat tca atg Phe Ser Val Gly Leu Phe Val Asn Arg Phe Gly Arg Arg Asn Ser Met 85 90 95	467
ctg atg atg aac ctg ctg gcc ttc gtg tcc gcc gtg ctc atg ggc ttc Leu Met Met Asn Leu Leu Ala Phe Val Ser Ala Val Leu Met Gly Phe 100 105 110	515
tcg aaa ctg ggc aag tcc ttt gag atg ctg atc ctg ggc cgc ttc atc Ser Lys Leu Gly Lys Ser Phe Glu Met Leu Ile Leu Gly Arg Phe Ile 115 120 125	563
atc ggt gtg tac tgc ggc ctg acc aca ggc ttc gtg ccc atg tat gtg Ile Gly Val Tyr Cys Gly Leu Thr Thr Gly Phe Val Pro Met Tyr Val 130 135 140	611
ggt gaa gtg tca ccc aca gcc ttt cgt ggg gcc ctg ggc acc ctg cac Gly Glu Val Ser Pro Thr Ala Phe Arg Gly Ala Leu Gly Thr Leu His 145 150 155 160	659
cag ctg ggc atc gtc gtc ggc atc ctc atc gcc cag gtg ttc ggc ctg Gln Leu Gly Ile Val Val Gly Ile Leu Ile Ala Gln Val Phe Gly Leu 165 170 175	707
gac tcc atc atg ggc aac aag gac ctg tgg ccc ctg ctg ctg agc atc Asp Ser Ile Met Gly Asn Lys Asp Leu Trp Pro Leu Leu Leu Ser Ile 180 185 190	755
atc ttc atc ccg gcc ctg ctg cag tgc atc gtg ctg ccc ttc tgc ccc Ile Phe Ile Pro Ala Leu Leu Gln Cys Ile Val Leu Pro Phe Cys Pro 195 200 205	803
gag agt ccc cgc ttc ctg ctc atc aac cgc aac gag gag aac cgg gcc Glu Ser Pro Arg Phe Leu Leu Ile Asn Arg Asn Glu Glu Asn Arg Ala 210 215 220	851
aag agt gtg cta aag aag ctg cgc ggg aca gct gac gtg acc cat gac Lys Ser Val Leu Lys Lys Leu Arg Gly Thr Ala Asp Val Thr His Asp 225 230 235 240	899
ctg cag gag atg aag gaa gag agt cgg cag atg atg cgg gag aag aag Leu Gln Glu Met Lys Glu Glu Ser Arg Gln Met Met Arg Glu Lys Lys 245 250 255	947
gtc acc atc ctg gag ctg ttc cgc tcc ccc gcc tac cgc cag ccc atc Val Thr Ile Leu Glu Leu Phe Arg Ser Pro Ala Tyr Arg Gln Pro Ile 260 265 270	995
ctc atc gct gtg gtg ctg cag ctg tcc cag cag ctg tct ggc atc aac Leu Ile Ala Val Val Leu Gln Leu Ser Gln Gln Leu Ser Gly Ile Asn 275 280 285	1043
gct gtc ttc tat tac tcc acg agc atc ttc gag aag gcg ggg gtg cag Ala Val Phe Tyr Tyr Ser Thr Ser Ile Phe Glu Lys Ala Gly Val Gln 290 295 300	1091
cag cct gtg tat gcc acc att ggc tcc ggt atc gtc aac acg gcc ttc Gln Pro Val Tyr Ala Thr Ile Gly Ser Gly Ile Val Asn Thr Ala Phe 305 310 315 320	1139
act gtc gtg tcg ctg ttt gtg gtg gag cga gca ggc cgg cgg acc ctg Thr Val Val Ser Leu Phe Val Val Glu Arg Ala Gly Arg Arg Thr Leu 325 330 335	1187

cac ctc ata ggc ctc gct ggc atg gcg ggt tgt gcc ata ctc atg acc His Leu Ile Gly Leu Ala Gly Met Ala Gly Cys Ala Ile Leu Met Thr 340 345 350	1235
atc gcg cta gca ctg ctg gag cag cta ccc tgg atg tcc tat ctg agc Ile Ala Leu Ala Leu Leu Glu Gln Leu Pro Trp Met Ser Tyr Leu Ser 355 360 365	1283
atc gtg gcc atc ttt ggc ttt gtg gcc ttc ttt gaa gtg ggt cct ggc Ile Val Ala Ile Phe Gly Phe Val Ala Phe Phe Glu Val Gly Pro Gly 370 375 380	1331
ccc atc cca tgg ttc atc gtg gct gaa ctc ttc agc cag ggt cca cgt Pro Ile Pro Trp Phe Ile Val Ala Glu Leu Phe Ser Gln Gly Pro Arg 385 390 395 400	1379
cca gct gcc att gcc gtt gca ggc ttc tcc aac tgg acc tca aat ttc Pro Ala Ala Ile Ala Val Ala Gly Phe Ser Asn Trp Thr Ser Asn Phe 405 410 415	1427
att gtg ggc atg tgc ttc cag tat gtg gag caa ctg tgt ggt ccc tac Ile Val Gly Met Cys Phe Gln Tyr Val Glu Gln Leu Cys Gly Pro Tyr 420 425 430	1475
gtc ttc atc atc ttc act gtg ctc ctg gtt ctg ttc ttc atc ttc acc Val Phe Ile Ile Phe Thr Val Leu Leu Val Leu Phe Phe Ile Phe Thr 435 440 445	1523
tac ttc aaa gtt cct gag act aaa ggc cgg acc ttc gat gag atc gct Tyr Phe Lys Val Pro Glu Thr Lys Gly Arg Thr Phe Asp Glu Ile Ala 450 455 460	1571
tcc ggc ttc cgg cag ggg gga gcc agc caa agt gat aag aca ccc gag Ser Gly Phe Arg Gln Gly Gly Ala Ser Gln Ser Asp Lys Thr Pro Glu 465 470 475 480	1619
gag ctg ttc cat ccc ctg ggg gct gat tcc caa gtg tga gtcgccccag Glu Leu Phe His Pro Leu Gly Ala Asp Ser Gln Val 485 490	1668
atcaccagcc cggcctgctc ccagcagccc taaggatctc tcaggagcac aggcagctgg	1728
atgagacttc caaacctgac agatgtcagc cgagccgggc ctggggctcc tttctccagc	1788
cagcaatgat gtccagaaga atattcagga cttaacggct ccaggatttt aacaaaagca	1848
agactgttgc tcaaattctat tcagacaagc aacaggtttt ataatttttt tattactgat	1908
tttgttattt ttatatcagc ctgagtctcc tgtgcccaca tcccaggctt caccctgaat	1968
ggttccatgc ctgagggttg agactaagcc ctgtcgagac acttgccctt ttcaccacgc	2028
taatctgtag ggctggacct atgtcctaag gacacactaa tcgaactatg aactacaaag	2088
cttctatccc aggaggtggc tatggccacc cgttctgctg gcctggatct cccactcta	2148
ggggtcaggc tccattagga ttgcccctt cccatctctt cctacccaac cactcaaatt	2208
aatctttctt taactgagac cagttggggag cactggagtg cagggaggag aggggaaggg	2268
ccagtctggg ctgccgggtt ctagtctcct ttgactgag ggccacacta ttaccatgag	2328

24

```

aagagggcct gtgggagcct gcaaactcac tgctcaagaa gacatggaga ctctgccct 2388
gttgtgtata gatgcaagat atttatatat attttttggt gtcaatatta aatacagaca 2448
ctaagttata gtatatctgg acaagccaac ttgtaaatac accacctcac tcctgttact 2508
tacctaaaca gatataaatg gctggttttt agaaacatgg ttttgaaatg cttgtggatt 2568
gagggtagga ggtttgatg ggagtgagac agaagtaagt ggggttgcaa ccaactgcaac 2628
ggcttagact tcgactcagg atccagtccc ttacacgtac ctctcatcag tgcctcttg 2688
ctcaaaaatc tgtttgatcc ctgttaccga gagaatatat acattcttta tcttgacatt 2748
caaggcattt ctatcacata tttgatagtt ggtgttcaaa aaaacactag ttttgtgcca 2808
gccgtgatgc tcaggcttga aatcgcatga ttttgaatgt gaagggaa 2856

```

```

<210> 11
<211> 492
<212> PRT
<213> GLUT1

```

```

<400> 11

```

```

Met Glu Pro Ser Ser Lys Lys Leu Thr Gly Arg Leu Met Leu Ala Val
1          5          10          15

```

```

Gly Gly Ala Val Leu Gly Ser Leu Gln Phe Gly Tyr Asn Thr Gly Val
          20          25          30

```

```

Ile Asn Ala Pro Gln Lys Val Ile Glu Glu Phe Tyr Asn Gln Thr Trp
          35          40          45

```

```

Val His Arg Tyr Gly Glu Ser Ile Leu Pro Thr Thr Leu Thr Thr Leu
          50          55          60

```

```

Trp Ser Leu Ser Val Ala Ile Phe Ser Val Gly Gly Met Ile Gly Ser
          65          70          75          80

```

```

Phe Ser Val Gly Leu Phe Val Asn Arg Phe Gly Arg Arg Asn Ser Met
          85          90          95

```

```

Leu Met Met Asn Leu Leu Ala Phe Val Ser Ala Val Leu Met Gly Phe
          100          105          110

```

```

Ser Lys Leu Gly Lys Ser Phe Glu Met Leu Ile Leu Gly Arg Phe Ile
          115          120          125

```

```

Ile Gly Val Tyr Cys Gly Leu Thr Thr Gly Phe Val Pro Met Tyr Val
          130          135          140

```

```

Gly Glu Val Ser Pro Thr Ala Phe Arg Gly Ala Leu Gly Thr Leu His

```

25

145		150		155		160									
Gln	Leu	Gly	Ile	Val	Val	Gly	Ile	Leu	Ile	Ala	Gln	Val	Phe	Gly	Leu
				165					170					175	
Asp	Ser	Ile	Met	Gly	Asn	Lys	Asp	Leu	Trp	Pro	Leu	Leu	Leu	Ser	Ile
			180					185						190	
Ile	Phe	Ile	Pro	Ala	Leu	Leu	Gln	Cys	Ile	Val	Leu	Pro	Phe	Cys	Pro
		195					200					205			
Glu	Ser	Pro	Arg	Phe	Leu	Leu	Ile	Asn	Arg	Asn	Glu	Glu	Asn	Arg	Ala
	210					215					220				
Lys	Ser	Val	Leu	Lys	Lys	Leu	Arg	Gly	Thr	Ala	Asp	Val	Thr	His	Asp
225					230					235					240
Leu	Gln	Glu	Met	Lys	Glu	Glu	Ser	Arg	Gln	Met	Met	Arg	Glu	Lys	Lys
				245					250					255	
Val	Thr	Ile	Leu	Glu	Leu	Phe	Arg	Ser	Pro	Ala	Tyr	Arg	Gln	Pro	Ile
			260					265					270		
Leu	Ile	Ala	Val	Val	Leu	Gln	Leu	Ser	Gln	Gln	Leu	Ser	Gly	Ile	Asn
		275					280					285			
Ala	Val	Phe	Tyr	Tyr	Ser	Thr	Ser	Ile	Phe	Glu	Lys	Ala	Gly	Val	Gln
	290					295					300				
Gln	Pro	Val	Tyr	Ala	Thr	Ile	Gly	Ser	Gly	Ile	Val	Asn	Thr	Ala	Phe
305					310					315					320
Thr	Val	Val	Ser	Leu	Phe	Val	Val	Glu	Arg	Ala	Gly	Arg	Arg	Thr	Leu
				325					330					335	
His	Leu	Ile	Gly	Leu	Ala	Gly	Met	Ala	Gly	Cys	Ala	Ile	Leu	Met	Thr
			340					345					350		
Ile	Ala	Leu	Ala	Leu	Leu	Glu	Gln	Leu	Pro	Trp	Met	Ser	Tyr	Leu	Ser
		355					360					365			
Ile	Val	Ala	Ile	Phe	Gly	Phe	Val	Ala	Phe	Phe	Glu	Val	Gly	Pro	Gly
	370					375					380				
Pro	Ile	Pro	Trp	Phe	Ile	Val	Ala	Glu	Leu	Phe	Ser	Gln	Gly	Pro	Arg
385					390					395					400

Pro Ala Ala Ile Ala Val Ala Gly Phe Ser Asn Trp Thr Ser Asn Phe
 405 410 415

Ile Val Gly Met Cys Phe Gln Tyr Val Glu Gln Leu Cys Gly Pro Tyr
 420 425 430

Val Phe Ile Ile Phe Thr Val Leu Leu Val Leu Phe Phe Ile Phe Thr
 435 440 445

Tyr Phe Lys Val Pro Glu Thr Lys Gly Arg Thr Phe Asp Glu Ile Ala
 450 455 460

Ser Gly Phe Arg Gln Gly Gly Ala Ser Gln Ser Asp Lys Thr Pro Glu
 465 470 475 480

Glu Leu Phe His Pro Leu Gly Ala Asp Ser Gln Val
 485 490

<210> 12
 <211> 1506
 <212> DNA
 <213> HA tagged GLUT1

<220>
 <221> CDS
 <222> (1)..(1506)
 <223>

<400> 12
 atg gag ccc agc agc aag aag ctg acg ggt cgc ctc atg ctg gct gtg 48
 Met Glu Pro Ser Ser Lys Lys Leu Thr Gly Arg Leu Met Leu Ala Val
 1 5 10 15
 gga gga gca gtg ctt ggc tcc ctg cag ttt ggc tac aac act gga gtc 96
 Gly Gly Ala Val Leu Gly Ser Leu Gln Phe Gly Tyr Asn Thr Gly Val
 20 25 30
 atc aat gcc ccc cag aag gtg atc gag gag ttc tac aac cag aca tgg 144
 Ile Asn Ala Pro Gln Lys Val Ile Glu Glu Phe Tyr Asn Gln Thr Trp
 35 40 45
 gtc cac cgc tat ggg gag agc atc tac cca tac gac gtc cca gac tac 192
 Val His Arg Tyr Gly Glu Ser Ile Tyr Pro Tyr Asp Val Pro Asp Tyr
 50 55 60
 gct ctg ccc acc acg ctc acc acg ctc tgg tcc ctc tca gtg gcc atc 240
 Ala Leu Pro Thr Thr Leu Thr Thr Leu Trp Ser Leu Ser Val Ala Ile
 65 70 75 80
 ttt tct gtt ggg ggc atg att ggc tcc ttc tct gtg ggc ctt ttc gtt 288
 Phe Ser Val Gly Gly Met Ile Gly Ser Phe Ser Val Gly Leu Phe Val
 85 90 95
 aac cgc ttt ggc cgg cgg aat tca atg ctg atg atg aac ctg ctg gcc 336
 Asn Arg Phe Gly Arg Arg Asn Ser Met Leu Met Met Asn Leu Leu Ala
 100 105 110

ttc gtg tcc gcc gtg ctc atg ggc ttc tcg aaa ctg ggc aag tcc ttt Phe Val Ser Ala Val Leu Met Gly Phe Ser Lys Leu Gly Lys Ser Phe 115 120 125	384
gag atg ctg atc ctg ggc cgc ttc atc atc ggt gtg tac tgc ggc ctg Glu Met Leu Ile Leu Gly Arg Phe Ile Ile Gly Val Tyr Cys Gly Leu 130 135 140	432
acc aca ggc ttc gtg ccc atg tat gtg ggt gaa gtg tca ccc aca gcc Thr Thr Gly Phe Val Pro Met Tyr Val Gly Glu Val Ser Pro Thr Ala 145 150 155 160	480
ttt cgt ggg gcc ctg ggc acc ctg cac cag ctg ggc atc gtc gtc ggc Phe Arg Gly Ala Leu Gly Thr Leu His Gln Leu Gly Ile Val Val Gly 165 170 175	528
atc ctc atc gcc cag gtg ttc ggc ctg gac tcc atc atg ggc aac aag Ile Leu Ile Ala Gln Val Phe Gly Leu Asp Ser Ile Met Gly Asn Lys 180 185 190	576
gac ctg tgg ccc ctg ctg ctg agc atc atc ttc atc ccg gcc ctg ctg Asp Leu Trp Pro Leu Leu Leu Ser Ile Ile Phe Ile Pro Ala Leu Leu 195 200 205	624
cag tgc atc gtg ctg ccc ttc tgc ccc gag agt ccc cgc ttc ctg ctc Gln Cys Ile Val Leu Pro Phe Cys Pro Glu Ser Pro Arg Phe Leu Leu 210 215 220	672
atc aac cgc aac gag gag aac cgg gcc aag agt gtg cta aag aag ctg Ile Asn Arg Asn Glu Glu Asn Arg Ala Lys Ser Val Leu Lys Lys Leu 225 230 235 240	720
cgc ggg aca gct gac gtg acc cat gac ctg cag gag atg aag gaa gag Arg Gly Thr Ala Asp Val Thr His Asp Leu Gln Glu Met Lys Glu Glu 245 250 255	768
agt cgg cag atg atg cgg gag aag aag gtc acc atc ctg gag ctg ttc Ser Arg Gln Met Met Arg Glu Lys Lys Val Thr Ile Leu Glu Leu Phe 260 265 270	816
cgc tcc ccc gcc tac cgc cag ccc atc ctc atc gct gtg gtg ctg cag Arg Ser Pro Ala Tyr Arg Gln Pro Ile Leu Ile Ala Val Val Leu Gln 275 280 285	864
ctg tcc cag cag ctg tct ggc atc aac gct gtc ttc tat tac tcc acg Leu Ser Gln Gln Leu Ser Gly Ile Asn Ala Val Phe Tyr Tyr Ser Thr 290 295 300	912
agc atc ttc gag aag gcg ggg gtg cag cag cct gtg tat gcc acc att Ser Ile Phe Glu Lys Ala Gly Val Gln Gln Pro Val Tyr Ala Thr Ile 305 310 315 320	960
ggc tcc ggt atc gtc aac acg gcc ttc act gtc gtg tcg ctg ttt gtg Gly Ser Gly Ile Val Asn Thr Ala Phe Thr Val Val Ser Leu Phe Val 325 330 335	1008
gtg gag cga gca ggc cgg cgg acc ctg cac ctc ata ggc ctc gct ggc Val Glu Arg Ala Gly Arg Arg Thr Leu His Leu Ile Gly Leu Ala Gly 340 345 350	1056
atg gcg ggt tgt gcc ata ctc atg acc atc gcg cta gca ctg ctg gag Met Ala Gly Cys Ala Ile Leu Met Thr Ile Ala Leu Ala Leu Leu Glu 355 360 365	1104

cag cta ccc tgg atg tcc tat ctg agc atc gtg gcc atc ttt ggc ttt 1152
 Gln Leu Pro Trp Met Ser Tyr Leu Ser Ile Val Ala Ile Phe Gly Phe
 370 375 380
 gtg gcc ttc ttt gaa gtg ggt cct ggc ccc atc cca tgg ttc atc gtg 1200
 Val Ala Phe Phe Glu Val Gly Pro Gly Pro Ile Pro Trp Phe Ile Val
 385 390 395 400
 gct gaa ctc ttc agc cag ggt cca cgt cca gct gcc att gcc gtt gca 1248
 Ala Glu Leu Phe Ser Gln Gly Pro Arg Pro Ala Ala Ile Ala Val Ala
 405 410 415
 ggc ttc tcc aac tgg acc tca aat ttc att gtg ggc atg tgc ttc cag 1296
 Gly Phe Ser Asn Trp Thr Ser Asn Phe Ile Val Gly Met Cys Phe Gln
 420 425 430
 tat gtg gag caa ctg tgt ggt ccc tac gtc ttc atc atc ttc act gtg 1344
 Tyr Val Glu Gln Leu Cys Gly Pro Tyr Val Phe Ile Ile Phe Thr Val
 435 440 445
 ctc ctg gtt ctg ttc ttc atc ttc acc tac ttc aaa gtt cct gag act 1392
 Leu Leu Val Leu Phe Phe Ile Phe Thr Tyr Phe Lys Val Pro Glu Thr
 450 455 460
 aaa ggc cgg acc ttc gat gag atc gct tcc ggc ttc cgg cag ggg gga 1440
 Lys Gly Arg Thr Phe Asp Glu Ile Ala Ser Gly Phe Arg Gln Gly Gly
 465 470 475 480
 gcc agc caa agt gat aag aca ccc gag gag ctg ttc cat ccc ctg ggg 1488
 Ala Ser Gln Ser Asp Lys Thr Pro Glu Glu Leu Phe His Pro Leu Gly
 485 490 495
 gct gat tcc caa gtg tga 1506
 Ala Asp Ser Gln Val
 500

<210> 13
 <211> 501
 <212> PRT
 <213> HA tagged GLUT1

<400> 13

Met Glu Pro Ser Ser Lys Lys Leu Thr Gly Arg Leu Met Leu Ala Val
1 5 10 15

Gly Gly Ala Val Leu Gly Ser Leu Gln Phe Gly Tyr Asn Thr Gly Val
20 25 30

Ile Asn Ala Pro Gln Lys Val Ile Glu Glu Phe Tyr Asn Gln Thr Trp
35 40 45

Val His Arg Tyr Gly Glu Ser Ile Tyr Pro Tyr Asp Val Pro Asp Tyr
50 55 60

Ala Leu Pro Thr Thr Leu Thr Thr Leu Trp Ser Leu Ser Val Ala Ile
65 70 75 80

Phe Ser Val Gly Gly Met Ile Gly Ser Phe Ser Val Gly Leu Phe Val
 85 90 95

Asn Arg Phe Gly Arg Arg Asn Ser Met Leu Met Met Asn Leu Leu Ala
 100 105 110

Phe Val Ser Ala Val Leu Met Gly Phe Ser Lys Leu Gly Lys Ser Phe
 115 120 125

Glu Met Leu Ile Leu Gly Arg Phe Ile Ile Gly Val Tyr Cys Gly Leu
 130 135 140

Thr Thr Gly Phe Val Pro Met Tyr Val Gly Glu Val Ser Pro Thr Ala
 145 150 155 160

Phe Arg Gly Ala Leu Gly Thr Leu His Gln Leu Gly Ile Val Val Gly
 165 170 175

Ile Leu Ile Ala Gln Val Phe Gly Leu Asp Ser Ile Met Gly Asn Lys
 180 185 190

Asp Leu Trp Pro Leu Leu Leu Ser Ile Ile Phe Ile Pro Ala Leu Leu
 195 200 205

Gln Cys Ile Val Leu Pro Phe Cys Pro Glu Ser Pro Arg Phe Leu Leu
 210 215 220

Ile Asn Arg Asn Glu Glu Asn Arg Ala Lys Ser Val Leu Lys Lys Leu
 225 230 235 240

Arg Gly Thr Ala Asp Val Thr His Asp Leu Gln Glu Met Lys Glu Glu
 245 250 255

Ser Arg Gln Met Met Arg Glu Lys Lys Val Thr Ile Leu Glu Leu Phe
 260 265 270

Arg Ser Pro Ala Tyr Arg Gln Pro Ile Leu Ile Ala Val Val Leu Gln
 275 280 285

Leu Ser Gln Gln Leu Ser Gly Ile Asn Ala Val Phe Tyr Tyr Ser Thr
 290 295 300

Ser Ile Phe Glu Lys Ala Gly Val Gln Gln Pro Val Tyr Ala Thr Ile
 305 310 315 320

Gly Ser Gly Ile Val Asn Thr Ala Phe Thr Val Val Ser Leu Phe Val

30

325 330 335
 Val Glu Arg Ala Gly Arg Arg Thr Leu His Leu Ile Gly Leu Ala Gly
 340 345 350
 Met Ala Gly Cys Ala Ile Leu Met Thr Ile Ala Leu Ala Leu Leu Glu
 355 360 365
 Gln Leu Pro Trp Met Ser Tyr Leu Ser Ile Val Ala Ile Phe Gly Phe
 370 375 380
 Val Ala Phe Phe Glu Val Gly Pro Gly Pro Ile Pro Trp Phe Ile Val
 385 390 395 400
 Ala Glu Leu Phe Ser Gln Gly Pro Arg Pro Ala Ala Ile Ala Val Ala
 405 410 415
 Gly Phe Ser Asn Trp Thr Ser Asn Phe Ile Val Gly Met Cys Phe Gln
 420 425 430
 Tyr Val Glu Gln Leu Cys Gly Pro Tyr Val Phe Ile Ile Phe Thr Val
 435 440 445
 Leu Leu Val Leu Phe Phe Ile Phe Thr Tyr Phe Lys Val Pro Glu Thr
 450 455 460
 Lys Gly Arg Thr Phe Asp Glu Ile Ala Ser Gly Phe Arg Gln Gly Gly
 465 470 475 480
 Ala Ser Gln Ser Asp Lys Thr Pro Glu Glu Leu Phe His Pro Leu Gly
 485 490 495
 Ala Asp Ser Gln Val
 500

<210> 14
 <211> 9
 <212> PRT
 <213> HA epitope

<400> 14

Tyr Pro Tyr Asp Val Pro Asp Tyr Ala
 1 5

<210> 15
 <211> 14
 <212> PRT
 <213> Simian Virus 5 epitope (SV5)

31

<400> 15

Gly Lys Pro Ile Pro Asn Pro Leu Leu Gly Leu Asp Ser Thr
1 5 10

<210> 16

<211> 6

<212> PRT

<213> hexa-his

<400> 16

His His His His His His
1 5

<210> 17

<211> 10

<212> PRT

<213> c-myc epitope

<400> 17

Phe Gln Lys Leu Ile Ser Glu Glu Asp Leu
1 5 10

<210> 18

<211> 9

<212> PRT

<213> FLAG epitope

<400> 18

Asp Tyr Lys Asp Asp Asp Asp Lys Cys
1 5

<210> 19

<211> 9

<212> PRT

<213> Alternative FLAG epitope

<400> 19

Met Asp Phe Lys Asp Asp Asp Asp Lys
1 5

<210> 20

<211> 9

<212> PRT

<213> Alternative FLAG epitope

<400> 20

Met Asp Tyr Lys Ala Phe Asp Asn Leu
1 5

<210> 21

<211> 223

32

<212> PRT

<213> glutathione-S-transferase

<400> 21

Met Ala Lys Leu Pro Ile Leu Gly Tyr Trp Lys Ile Lys Gly Leu Val
 1 5 10 15

Gln Pro Thr Arg Leu Leu Leu Glu Tyr Leu Glu Glu Lys Tyr Glu Glu
 20 25 30

His Leu Tyr Glu Arg Asp Glu Gly Asp Lys Trp Arg Asn Lys Lys Phe
 35 40 45

Glu Leu Gly Leu Glu Phe Pro Asn Leu Pro Tyr Tyr Ile Asp Gly Asp
 50 55 60

Val Lys Leu Thr Gln Ser Met Ala Ile Ile Arg Tyr Ile Ala Asp Lys
 65 70 75 80

His Asn Met Leu Gly Gly Cys Pro Lys Glu Arg Ala Glu Ile Ser Met
 85 90 95

Leu Glu Gly Ala Val Leu Asp Ile Arg Tyr Gly Val Ser Arg Ile Ala
 100 105 110

Tyr Ser Lys Asp Phe Glu Thr Leu Lys Val Asp Phe Leu Ser Lys Leu
 115 120 125

Pro Glu Met Leu Lys Met Phe Glu Asp Arg Leu Cys His Lys Thr Tyr
 130 135 140

Leu Asn Gly Asp His Val Thr His Pro Asp Phe Met Leu Tyr Asp Ala
 145 150 155 160

Leu Asp Val Val Leu Tyr Met Asp Pro Met Cys Leu Asp Ala Phe Pro
 165 170 175

Lys Leu Val Cys Phe Lys Lys Arg Ile Glu Ala Ile Pro Gln Ile Asp
 180 185 190

Lys Tyr Leu Lys Ser Ser Lys Tyr Ile Ala Trp Pro Leu Gln Gly Trp
 195 200 205

Gln Ala Thr Phe Gly Gly Gly Asp His Pro Pro Lys Ser Asp Leu
 210 215 220

<210> 22

<211> 488

<212> PRT

<213> maltose binding protein

<400> 22

Met Lys Ile Glu Glu Gly Lys Leu Val Ile Trp Ile Asn Gly Asp Lys
 1 5 10 15

Gly Tyr Asn Gly Leu Ala Glu Val Gly Lys Lys Phe Glu Lys Asp Thr
 20 25 30

Gly Ile Lys Val Thr Val Glu His Pro Asp Lys Leu Glu Glu Lys Phe
 35 40 45

Pro Gln Val Ala Ala Thr Gly Asp Gly Pro Asp Ile Ile Phe Trp Ala
 50 55 60

His Asp Arg Phe Gly Gly Tyr Ala Gln Ser Gly Leu Leu Ala Glu Ile
 65 70 75 80

Thr Pro Asp Lys Ala Phe Gln Asp Lys Leu Tyr Pro Phe Thr Trp Asp
 85 90 95

Ala Val Arg Tyr Asn Gly Lys Leu Ile Ala Tyr Pro Ile Ala Val Glu
 100 105 110

Ala Leu Ser Leu Ile Tyr Asn Lys Asp Leu Leu Pro Asn Pro Pro Lys
 115 120 125

Thr Trp Glu Glu Ile Pro Ala Leu Asp Lys Glu Leu Lys Ala Lys Gly
 130 135 140

Lys Ser Ala Leu Met Phe Asn Leu Gln Glu Pro Tyr Phe Thr Trp Pro
 145 150 155 160

Leu Ile Ala Ala Asp Gly Gly Tyr Ala Phe Lys Tyr Glu Asn Gly Lys
 165 170 175

Tyr Asp Ile Lys Asp Val Gly Val Asp Asn Ala Gly Ala Lys Ala Gly
 180 185 190

Leu Thr Phe Leu Val Asp Leu Ile Lys Asn Lys His Met Asn Ala Asp
 195 200 205

Thr Asp Tyr Ser Ile Ala Glu Ala Ala Phe Asn Lys Gly Glu Thr Ala
 210 215 220

Met Thr Ile Asn Gly Pro Trp Ala Trp Ser Asn Ile Asp Thr Ser Lys
 225 230 235 240

Val Asn Tyr Gly Val Thr Val Leu Pro Thr Phe Lys Gly Gln Pro Ser
245 250 255

Lys Pro Phe Val Gly Val Leu Ser Ala Gly Ile Asn Ala Ala Ser Pro
260 265 270

Asn Lys Glu Leu Ala Lys Glu Phe Leu Glu Asn Tyr Leu Leu Thr Asp
275 280 285

Glu Gly Leu Glu Ala Val Asn Lys Asp Lys Pro Leu Gly Ala Val Ala
290 295 300

Leu Lys Ser Tyr Glu Glu Glu Leu Ala Lys Asp Pro Arg Ile Ala Ala
305 310 315 320

Thr Met Glu Asn Ala Gln Lys Gly Glu Ile Met Pro Asn Ile Pro Gln
325 330 335

Met Ser Ala Phe Trp Tyr Ala Val Arg Thr Ala Val Ile Asn Ala Ala
340 345 350

Ser Gly Arg Gln Thr Val Asp Glu Ala Leu Lys Asp Ala Gln Thr Asn
355 360 365

Ser Ser Ser Asn Asn Asn Asn Asn Asn Asn Asn Asn Asn Leu Gly Ile
370 375 380

Asp Thr Thr Glu Asn Leu Tyr Phe Gln Gly Ala Met Asp Pro Glu Phe
385 390 395 400

Lys Gly Leu Arg Arg Arg Ala Gln Leu Val Arg Pro Leu Ser Asn Leu
405 410 415

Glu Pro Ala Val Ser Arg His Ala Val Pro Ser Leu Ala Leu Ala Val
420 425 430

Val Leu Gln Arg Arg Asp Trp Glu Asn Pro Gly Val Thr Gln Leu Asn
435 440 445

Arg Leu Ala Ala His Pro Pro Phe Ala Ser Trp Arg Asn Ser Glu Glu
450 455 460

Ala Arg Thr Asp Arg Pro Ser Gln Gln Leu Arg Ser Leu Asn Gly Glu
465 470 475 480

Trp Gln Leu Gly Cys Phe Gly Gly

35

485

<210> 23
 <211> 168
 <212> PRT
 <213> GAL4

<400> 23

Met Lys Leu Leu Ser Ser Ile Glu Gln Ala Cys Asp Ile Cys Arg Leu
 1 5 10 15

Lys Lys Leu Lys Cys Ser Lys Glu Lys Pro Lys Cys Ala Lys Cys Leu
 20 25 30

Lys Asn Asn Trp Glu Cys Arg Tyr Ser Pro Lys Thr Lys Arg Ser Pro
 35 40 45

Leu Thr Arg Ala His Leu Thr Glu Val Glu Ser Arg Leu Glu Arg Leu
 50 55 60

Glu Gln Leu Phe Leu Leu Ile Phe Pro Arg Glu Asp Leu Asp Met Ile
 65 70 75 80

Leu Lys Met Asp Ser Leu Gln Asp Ile Lys Ala Leu Leu Thr Gly Leu
 85 90 95

Phe Val Gln Asp Asn Val Asn Lys Asp Ala Val Thr Asp Arg Leu Ala
 100 105 110

Ser Val Glu Thr Asp Met Pro Leu Thr Leu Arg Gln His Arg Ile Ser
 115 120 125

Ala Thr Ser Ser Ser Glu Glu Ser Ser Asn Lys Gly Gln Arg Gln Leu
 130 135 140

Thr Val Ser Pro Glu Phe Pro Gly Ile Arg Arg Leu Asp Ala Leu Ile
 145 150 155 160

Ser Ser Arg Ala Ala Ala Gly Thr
 165

<210> 24
 <211> 1045
 <212> PRT
 <213> beta-galactosidase

<400> 24

Met Ser Phe Thr Leu Thr Asn Lys Asn Val Ile Phe Val Ala Gly Leu
 1 5 10 15

Gly Gly Ile Gly Leu Asp Thr Ser Lys Glu Leu Leu Lys Arg Asp Pro
 20 25 30

Val Val Leu Gln Arg Arg Asp Trp Glu Asn Pro Gly Val Thr Gln Leu
 35 40 45

Asn Arg Leu Ala Ala His Pro Pro Phe Ala Ser Trp Arg Asn Ser Glu
 50 55 60

Glu Ala Arg Thr Asp Arg Pro Ser Gln Gln Leu Arg Ser Leu Asn Gly
 65 70 75 80

Glu Trp Arg Phe Ala Trp Phe Pro Ala Pro Glu Ala Val Pro Glu Ser
 85 90 95

Trp Leu Glu Cys Asp Leu Pro Glu Ala Asp Thr Val Val Val Pro Ser
 100 105 110

Asn Trp Gln Met His Gly Tyr Asp Ala Pro Ile Tyr Thr Asn Val Thr
 115 120 125

Tyr Pro Ile Thr Val Asn Pro Pro Phe Val Pro Thr Glu Asn Pro Thr
 130 135 140

Gly Cys Tyr Ser Leu Thr Phe Asn Val Asp Glu Ser Trp Leu Gln Glu
 145 150 155 160

Gly Gln Thr Arg Ile Ile Phe Asp Gly Val Asn Ser Ala Phe His Leu
 165 170 175

Trp Cys Asn Gly Arg Trp Val Gly Tyr Gly Gln Asp Ser Arg Leu Pro
 180 185 190

Ser Glu Phe Asp Leu Ser Ala Phe Leu Arg Ala Gly Glu Asn Arg Leu
 195 200 205

Ala Val Met Val Leu Arg Trp Ser Asp Gly Ser Tyr Leu Glu Asp Gln
 210 215 220

Asp Met Trp Arg Met Ser Gly Ile Phe Arg Asp Val Ser Leu Leu His
 225 230 235 240

Lys Pro Thr Thr Gln Ile Ser Asp Phe His Val Ala Thr Arg Phe Asn
 245 250 255

Asp Asp Phe Ser Arg Ala Val Leu Glu Ala Glu Val Gln Met Cys Gly

260	265	270
Glu Leu Arg Asp Tyr Leu Arg Val Thr Val Ser Leu Trp Gln Gly Glu		
275	280	285
Thr Gln Val Ala Ser Gly Thr Ala Pro Phe Gly Gly Glu Ile Ile Asp		
290	295	300
Glu Arg Gly Gly Tyr Ala Asp Arg Val Thr Leu Arg Leu Asn Val Glu		
305	310	315
Asn Pro Lys Leu Trp Ser Ala Glu Ile Pro Asn Leu Tyr Arg Ala Val		
325	330	335
Val Glu Leu His Thr Ala Asp Gly Thr Leu Ile Glu Ala Glu Ala Cys		
340	345	350
Asp Val Gly Phe Arg Glu Val Arg Ile Glu Asn Gly Leu Leu Leu Leu		
355	360	365
Asn Gly Lys Pro Leu Leu Ile Arg Gly Val Asn Arg His Glu His His		
370	375	380
Pro Leu His Gly Gln Val Met Asp Glu Gln Thr Met Val Gln Asp Ile		
385	390	395
Leu Leu Met Lys Gln Asn Asn Phe Asn Ala Val Arg Cys Ser His Tyr		
405	410	415
Pro Asn His Pro Leu Trp Tyr Thr Leu Cys Asp Arg Tyr Gly Leu Tyr		
420	425	430
Val Val Asp Glu Ala Asn Ile Glu Thr His Gly Met Val Pro Met Asn		
435	440	445
Arg Leu Thr Asp Asp Pro Arg Trp Leu Pro Ala Met Ser Glu Arg Val		
450	455	460
Thr Arg Met Val Gln Arg Asp Arg Asn His Pro Ser Val Ile Ile Trp		
465	470	475
Ser Leu Gly Asn Glu Ser Gly His Gly Ala Asn His Asp Ala Leu Tyr		
485	490	495
Arg Trp Ile Lys Ser Val Asp Pro Ser Arg Pro Val Gln Tyr Glu Gly		
500	505	510

Gly Gly Ala Asp Thr Thr Ala Thr Asp Ile Ile Cys Pro Met Tyr Ala
515 520 525

Arg Val Asp Glu Asp Gln Pro Phe Pro Ala Val Pro Lys Trp Ser Ile
530 535 540

Lys Lys Trp Leu Ser Leu Pro Gly Glu Thr Arg Pro Leu Ile Leu Cys
545 550 555 560

Glu Tyr Ala His Ala Met Gly Asn Ser Leu Gly Gly Phe Ala Lys Tyr
565 570 575

Trp Gln Ala Phe Arg Gln Tyr Pro Arg Leu Gln Gly Gly Phe Val Trp
580 585 590

Asp Trp Val Asp Gln Ser Leu Ile Lys Tyr Asp Glu Asn Gly Asn Pro
595 600 605

Trp Ser Ala Tyr Gly Gly Asp Phe Gly Asp Thr Pro Asn Asp Arg Gln
610 615 620

Phe Cys Met Asn Gly Leu Val Phe Ala Asp Arg Thr Pro His Pro Ala
625 630 635 640

Leu Thr Glu Ala Lys His Gln Gln Gln Phe Phe Gln Phe Arg Leu Ser
645 650 655

Gly Gln Thr Ile Glu Val Thr Ser Glu Tyr Leu Phe Arg His Ser Asp
660 665 670

Asn Glu Leu Leu His Trp Met Val Ala Leu Asp Gly Lys Pro Leu Ala
675 680 685

Ser Gly Glu Val Pro Leu Asp Val Ala Pro Gln Gly Lys Gln Leu Ile
690 695 700

Glu Leu Pro Glu Leu Pro Gln Pro Glu Ser Ala Gly Gln Leu Trp Leu
705 710 715 720

Thr Val Arg Val Val Gln Pro Asn Ala Thr Ala Trp Ser Glu Ala Gly
725 730 735

His Ile Ser Ala Trp Gln Gln Trp Arg Leu Ala Glu Asn Leu Ser Val
740 745 750

Thr Leu Pro Ala Ala Ser His Ala Ile Pro His Leu Thr Thr Ser Glu
755 760 765

Met Asp Phe Cys Ile Glu Leu Gly Asn Lys Arg Trp Gln Phe Asn Arg
 770 775 780

Gln Ser Gly Phe Leu Ser Gln Met Trp Ile Gly Asp Lys Lys Gln Leu
 785 790 795 800

Leu Thr Pro Leu Arg Asp Gln Phe Thr Arg Ala Pro Leu Asp Asn Asp
 805 810 815

Ile Gly Val Ser Glu Ala Thr Arg Ile Asp Pro Asn Ala Trp Val Glu
 820 825 830

Arg Trp Lys Ala Ala Gly His Tyr Gln Ala Glu Ala Ala Leu Leu Gln
 835 840 845

Cys Thr Ala Asp Thr Leu Ala Asp Ala Val Leu Ile Thr Thr Ala His
 850 855 860

Ala Trp Gln His Gln Gly Lys Thr Leu Phe Ile Ser Arg Lys Thr Tyr
 865 870 875 880

Arg Ile Asp Gly Ser Gly Gln Met Ala Ile Thr Val Asp Val Glu Val
 885 890 895

Ala Ser Asp Thr Pro His Pro Ala Arg Ile Gly Leu Asn Cys Gln Leu
 900 905 910

Ala Gln Val Ala Glu Arg Val Asn Trp Leu Gly Leu Gly Pro Gln Glu
 915 920 925

Asn Tyr Pro Asp Arg Leu Thr Ala Ala Cys Phe Asp Arg Trp Asp Leu
 930 935 940

Pro Leu Ser Asp Met Tyr Thr Pro Tyr Val Phe Pro Ser Glu Asn Gly
 945 950 955 960

Leu Arg Cys Gly Thr Arg Glu Leu Asn Tyr Gly Pro His Gln Trp Arg
 965 970 975

Gly Asp Phe Gln Phe Asn Ile Ser Arg Tyr Ser Gln Gln Gln Leu Met
 980 985 990

Ser His Arg His Leu Leu His Ala Glu Glu Gly Thr Trp Leu Asn Ile
 995 1000 1005

Asp Gly Phe His Met Gly Ile Gly Gly Asp Asp Ser Trp Ser Pro
 1010 1015 1020

Ser Val Ser Ala Glu Leu Gln Leu Ser Ala Gly Arg Tyr His Tyr
 1025 1030 1035

Gln Leu Val Trp Cys Gln Lys
 1040 1045

<210> 25
 <211> 238
 <212> PRT
 <213> enhanced green fluorescence protein (eGFP)

<400> 25

Met Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val
 1 5 10 15

Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu
 20 25 30

Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile Cys
 35 40 45

Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Phe
 50 55 60

Gly Tyr Gly Val Gln Cys Phe Ala Arg Tyr Pro Asp His Met Lys Gln
 65 70 75 80

His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg
 85 90 95

Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val
 100 105 110

Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile
 115 120 125

Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn
 130 135 140

Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn Gly
 145 150 155 160

Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser Val
 165 170 175

Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro
 180 185 190

Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu Ser
 195 200 205

Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe Val
 210 215 220

Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu Leu Tyr Lys
 225 230 235

<210> 26
 <211> 264
 <212> PRT
 <213> yellow fluorescent protein

<400> 26

Met Asp Gly Thr Glu Leu Gly Ser Thr Arg Asp Ser Arg Gly Ser Gly
 1 5 10 15

Gly Ser Gly Gly Ser Gly Gly Ser Gly Met Val Ser Lys Gly Glu Glu
 20 25 30

Leu Phe Thr Gly Val Val Pro Ile Leu Val Glu Leu Asp Gly Asp Val
 35 40 45

Asn Gly His Lys Phe Ser Val Ser Gly Glu Gly Glu Gly Asp Ala Thr
 50 55 60

Tyr Gly Lys Leu Thr Leu Lys Phe Ile Cys Thr Thr Gly Lys Leu Pro
 65 70 75 80

Val Pro Trp Pro Thr Leu Val Thr Thr Phe Gly Tyr Gly Leu Gln Cys
 85 90 95

Phe Ala Arg Tyr Pro Asp His Met Lys Gln His Asp Phe Phe Lys Ser
 100 105 110

Ala Met Pro Glu Gly Tyr Val Gln Glu Arg Thr Ile Phe Phe Lys Asp
 115 120 125

Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val Lys Phe Glu Gly Asp Thr
 130 135 140

Leu Val Asn Arg Ile Glu Leu Lys Gly Ile Asp Phe Lys Glu Asp Gly
 145 150 155 160

Asn Ile Leu Gly His Lys Leu Glu Tyr Asn Tyr Asn Ser His Asn Val
 165 170 175

Tyr Ile Met Ala Asp Lys Gln Lys Asn Gly Ile Lys Val Asn Phe Lys
 180 185 190

Ile Arg His Asn Ile Glu Asp Gly Ser Val Gln Leu Ala Asp His Tyr
 195 200 205

Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro Val Leu Leu Pro Asp Asn
 210 215 220

His Tyr Leu Ser Tyr Gln Ser Ala Leu Ser Lys Asp Pro Asn Glu Lys
 225 230 235 240

Arg Asp His Met Val Leu Leu Glu Phe Val Thr Ala Ala Gly Ile Thr
 245 250 255

Leu Gly Met Asp Glu Leu Tyr Lys
 260

<210> 27
 <211> 238
 <212> PRT
 <213> soluble modified blue fluorescent protein

<400> 27

Met Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val
 1 5 10 15

Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu
 20 25 30

Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile Cys
 35 40 45

Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Phe
 50 55 60

Ser His Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys Arg
 65 70 75 80

His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg
 85 90 95

Thr Ile Ser Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val
 100 105 110

Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile
 115 120 125

Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn
 130 135 140

Tyr Asn Ser His Asn Val Tyr Ile Thr Ala Asp Lys Gln Lys Asn Gly
 145 150 155 160

Ile Lys Ala Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser Val
 165 170 175

Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro
 180 185 190

Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu Ser
 195 200 205

Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe Val
 210 215 220

Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu Leu Tyr Lys
 225 230 235

<210> 28

<211> 238

<212> PRT

<213> soluble-modified red-shifted green fluorescent protein

<400> 28

Met Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val
 1 5 10 15

Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu
 20 25 30

Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile Cys
 35 40 45

Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Phe
 50 55 60

Thr Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys Arg
 65 70 75 80

His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg
 85 90 95

Thr Ile Ser Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val
 100 105 110

Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile
 115 120 125

Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn
 130 135 140

Tyr Asn Ser His Asn Val Tyr Ile Thr Ala Asp Lys Gln Lys Asn Gly
 145 150 155 160

Ile Lys Ala Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser Val
 165 170 175

Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro
 180 185 190

Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu Ser
 195 200 205

Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe Val
 210 215 220

Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu Leu Tyr Lys
 225 230 235

<210> 29
 <211> 262
 <212> PRT
 <213> cyan fluorescent protein

<400> 29

Met His His His His His His His Asp Gly Thr Met Val Ser Lys Gly
 1 5 10 15

Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val Glu Leu Asp Gly
 20 25 30

Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu Gly Glu Gly Asp
 35 40 45

Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile Cys Thr Thr Gly Lys
 50 55 60

Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Leu Thr Trp Gly Val
 65 70 75 80

Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys Gln His Asp Phe Phe
 85 90 95

45

Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg Thr Ile Phe Phe
 100 105 110

Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val Lys Phe Glu Gly
 115 120 125

Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile Asp Phe Lys Glu
 130 135 140

Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn Tyr Ile Ser His
 145 150 155 160

Asn Val Tyr Ile Thr Ala Asp Lys Gln Lys Asn Gly Ile Lys Ala Asn
 165 170 175

Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser Val Gln Leu Ala Asp
 180 185 190

His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro Val Leu Leu Pro
 195 200 205

Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu Ser Lys Asp Pro Asn
 210 215 220

Glu Lys Arg Asp His Met Val Leu Leu Glu Phe Val Thr Ala Ala Gly
 225 230 235 240

Ile Thr Leu Gly Met Asp Glu Leu Tyr Ser Gly Ser Gly Ser Gly Ser
 245 250 255

Leu Glu Gly Thr Glu Leu
 260

<210> 30
 <211> 8
 <212> PRT
 <213> streptavidin binding sequence

<400> 30

Trp Ser His Pro Gln Phe Glu Lys
 1 5

<210> 31
 <211> 574
 <212> PRT
 <213> strepsolysin-O

<400> 31

Met Lys Asp Met Ser Asn Lys Lys Thr Phe Lys Lys Tyr Ser Arg Val
 1 5 10 15
 Ala Gly Leu Leu Thr Ala Ala Leu Ile Ile Gly Asn Leu Val Thr Ala
 20 25 30
 Asn Ala Glu Ser Asn Lys Gln Asn Thr Ala Ser Thr Glu Thr Thr Thr
 35 40 45
 Thr Asn Glu Gln Pro Lys Pro Glu Ser Ser Glu Leu Thr Thr Glu Lys
 50 55 60
 Ala Gly Gln Lys Thr Asp Asp Met Leu Asn Ser Asn Asp Met Ile Lys
 65 70 75 80
 Leu Ala Pro Lys Glu Met Pro Leu Glu Ser Ala Glu Lys Glu Glu Lys
 85 90 95
 Lys Ser Glu Asp Lys Lys Lys Ser Glu Glu Asp His Thr Glu Glu Ile
 100 105 110
 Asn Asp Lys Ile Tyr Ser Leu Asn Tyr Asn Glu Leu Glu Val Leu Ala
 115 120 125
 Lys Asn Gly Glu Thr Ile Glu Asn Phe Val Pro Lys Glu Gly Val Lys
 130 135 140
 Lys Ala Asp Lys Phe Ile Val Ile Glu Arg Lys Lys Lys Asn Ile Asn
 145 150 155 160
 Thr Thr Pro Val Asp Ile Ser Ile Ile Asp Ser Val Thr Asp Arg Thr
 165 170 175
 Tyr Pro Ala Ala Leu Gln Leu Ala Asn Lys Gly Phe Thr Glu Asn Lys
 180 185 190
 Pro Asp Ala Val Val Thr Lys Arg Asn Pro Gln Lys Ile His Ile Asp
 195 200 205
 Leu Pro Gly Met Gly Asp Lys Ala Thr Val Glu Val Asn Asp Pro Thr
 210 215 220
 Tyr Ala Asn Val Ser Thr Ala Ile Asp Asn Leu Val Asn Gln Trp His
 225 230 235 240
 Asp Asn Tyr Ser Gly Gly Asn Thr Leu Pro Ala Arg Thr Gln Tyr Thr
 245 250 255

Glu Ser Met Val Tyr Ser Lys Ser Gln Ile Glu Ala Ala Leu Asn Val
 260 265 270

Asn Ser Lys Ile Leu Asp Gly Thr Leu Gly Ile Asp Phe Lys Ser Ile
 275 280 285

Ser Lys Gly Glu Lys Lys Val Met Ile Ala Ala Tyr Lys Gln Ile Phe
 290 295 300

Tyr Thr Val Ser Ala Asn Leu Pro Asn Asn Pro Ala Asp Val Phe Asp
 305 310 315 320

Lys Ser Val Thr Phe Lys Glu Leu Gln Arg Lys Gly Val Ser Asn Glu
 325 330 335

Ala Pro Pro Leu Phe Val Ser Asn Val Ala Tyr Gly Arg Thr Val Phe
 340 345 350

Val Lys Leu Glu Thr Ser Ser Lys Ser Asn Asp Val Glu Ala Ala Phe
 355 360 365

Ser Ala Ala Leu Lys Gly Thr Asp Val Lys Thr Asn Gly Lys Tyr Ser
 370 375 380

Asp Ile Leu Glu Asn Ser Ser Phe Thr Ala Val Val Leu Gly Gly Asp
 385 390 395 400

Ala Ala Glu His Asn Lys Val Val Thr Lys Asp Phe Asp Val Ile Arg
 405 410 415

Asn Val Ile Lys Asp Asn Ala Thr Phe Ser Arg Lys Asn Pro Ala Tyr
 420 425 430

Pro Ile Ser Tyr Thr Ser Val Phe Leu Lys Asn Asn Lys Ile Ala Gly
 435 440 445

Val Asn Asn Arg Thr Glu Tyr Val Glu Thr Thr Ser Thr Glu Tyr Thr
 450 455 460

Ser Gly Lys Ile Asn Leu Ser His Arg Gly Ala Tyr Val Ala Gln Tyr
 465 470 475 480

Glu Ile Leu Trp Asp Glu Ile Asn Tyr Asp Asp Lys Gly Lys Glu Val
 485 490 495

Ile Thr Lys Arg Arg Trp Asp Asn Asn Trp Tyr Ser Lys Thr Ser Pro

48

500 505 510
 Phe Ser Thr Val Ile Pro Leu Gly Ala Asn Ser Arg Asn Ile Arg Ile
 515 520 525
 Met Ala Arg Glu Cys Thr Gly Leu Ala Trp Glu Trp Trp Arg Lys Val
 530 535 540
 Ile Asp Glu Arg Asp Val Lys Leu Ser Lys Glu Ile Asn Val Asn Ile
 545 550 555 560
 Ser Gly Ser Thr Leu Ser Pro Tyr Gly Ser Ile Thr Tyr Lys
 565 570

 <210> 32
 <211> 293
 <212> PRT
 <213> alpha-hemolysin

 <400> 32

 Ala Asp Ser Asp Ile Asn Ile Lys Thr Gly Thr Thr Asp Ile Gly Ser
 1 5 10 15

 Asn Thr Thr Val Lys Thr Gly Asp Leu Val Thr Tyr Asp Lys Glu Asn
 20 25 30

 Gly Met His Lys Lys Val Phe Tyr Ser Phe Ile Asp Asp Lys Asn His
 35 40 45

 Asn Lys Lys Leu Leu Val Ile Arg Thr Lys Gly Thr Ile Ala Gly Gln
 50 55 60

 Tyr Arg Val Tyr Ser Glu Glu Gly Ala Asn Lys Ser Gly Leu Ala Trp
 65 70 75 80

 Pro Ser Ala Phe Lys Val Gln Leu Gln Leu Pro Asp Asn Glu Val Ala
 85 90 95

 Gln Ile Ser Asp Tyr Tyr Pro Arg Asn Ser Ile Asp Thr Lys Glu Tyr
 100 105 110

 Met Ser Thr Leu Thr Tyr Gly Phe Asn Gly Asn Val Thr Gly Asp Asp
 115 120 125

 Thr Gly Lys Ile Gly Gly Leu Ile Gly Ala Asn Val Ser Ile Gly His
 130 135 140

 Thr Leu Lys Tyr Val Gln Pro Asp Phe Lys Thr Ile Leu Glu Ser Pro

49

145 150 155 160
 Thr Asp Lys Lys Val Gly Trp Lys Val Ile Phe Asn Asn Met Val Asn
 165 170 175
 Gln Asn Trp Gly Pro Tyr Asp Arg Asp Ser Trp Asn Pro Val Tyr Gly
 180 185 190
 Asn Gln Leu Phe Met Lys Thr Arg Asn Gly Ser Met Lys Ala Ala Asp
 195 200 205
 Asn Phe Leu Asp Pro Asn Lys Ala Ser Ser Leu Leu Ser Ser Gly Phe
 210 215 220
 Ser Pro Asp Phe Ala Thr Val Ile Thr Met Asp Arg Lys Ala Ser Lys
 225 230 235 240
 Gln Gln Thr Asn Ile Asp Val Ile Tyr Glu Arg Val Arg Asp Asp Tyr
 245 250 255
 Gln Leu His Trp Thr Ser Thr Asn Trp Lys Gly Thr Asn Thr Lys Asp
 260 265 270
 Lys Trp Thr Asp Arg Ser Ser Glu Arg Tyr Lys Ile Asp Trp Glu Lys
 275 280 285
 Glu Glu Met Thr Asn
 290

 <210> 33
 <211> 527
 <212> PRT
 <213> tetanolysin-O

 <400> 33
 Met Asn Lys Asn Val Leu Lys Phe Val Ser Arg Ser Leu Leu Ile Phe
 1 5 10 15

 Ser Met Thr Gly Leu Ile Ser Asn Tyr Asn Ser Ser Asn Val Leu Ala
 20 25 30

 Lys Gly Asn Val Glu Glu His Ser Leu Ile Asn Asn Gly Gln Val Val
 35 40 45

 Thr Ser Asn Thr Lys Cys Asn Leu Ala Lys Asp Asn Ser Ser Asp Ile
 50 55 60

 Asp Lys Asn Ile Tyr Gly Leu Ser Tyr Asp Pro Arg Lys Ile Leu Ser

50

65		70		75		80									
Tyr	Asn	Gly	Glu	Gln	Val	Glu	Asn	Phe	Val	Pro	Ala	Glu	Gly	Phe	Glu
				85					90					95	
Asn	Pro	Asp	Lys	Phe	Ile	Val	Val	Lys	Arg	Glu	Lys	Lys	Ser	Ile	Ser
			100					105					110		
Asp	Ser	Thr	Ala	Asp	Ile	Ser	Ile	Ile	Asp	Ser	Ile	Asn	Asp	Arg	Thr
		115					120					125			
Tyr	Pro	Gly	Ala	Ile	Gln	Leu	Ala	Asn	Arg	Asn	Leu	Met	Glu	Asn	Lys
	130					135					140				
Pro	Asp	Ile	Ile	Ser	Cys	Glu	Arg	Lys	Pro	Ile	Thr	Ile	Ser	Val	Asp
145					150					155					160
Leu	Pro	Gly	Met	Ala	Glu	Asp	Gly	Lys	Lys	Val	Val	Asn	Ser	Pro	Thr
				165					170					175	
Tyr	Ser	Ser	Val	Asn	Ser	Ala	Ile	Asn	Ser	Ile	Leu	Asp	Thr	Trp	Asn
			180					185						190	
Ser	Lys	Tyr	Ser	Ser	Lys	Tyr	Thr	Ile	Pro	Thr	Arg	Met	Ser	Tyr	Ser
		195					200					205			
Asp	Thr	Met	Val	Tyr	Ser	Gln	Ser	Gln	Leu	Ser	Ala	Ala	Val	Gly	Cys
	210					215					220				
Asn	Phe	Lys	Ala	Leu	Asn	Lys	Ala	Leu	Asn	Ile	Asp	Phe	Asp	Ser	Ile
225					230					235					240
Phe	Lys	Gly	Glu	Lys	Lys	Val	Met	Leu	Leu	Ala	Tyr	Lys	Gln	Ile	Phe
				245					250					255	
Tyr	Thr	Val	Ser	Val	Asp	Pro	Pro	Asn	Arg	Pro	Ser	Asp	Leu	Phe	Gly
		260						265					270		
Asp	Ser	Val	Thr	Phe	Asp	Glu	Leu	Ala	Leu	Lys	Gly	Ile	Asn	Asn	Asn
		275					280					285			
Asn	Pro	Pro	Ala	Tyr	Val	Ser	Asn	Val	Ala	Tyr	Gly	Arg	Thr	Ile	Tyr
	290					295					300				
Val	Lys	Leu	Glu	Thr	Thr	Ser	Lys	Ser	Ser	His	Val	Lys	Ala	Ala	Phe
305					310					315					320

51

Lys Ala Leu Ile Asn Asn Gln Asp Ile Ser Ser Asn Ala Glu Tyr Lys
 325 330 335

Asp Ile Leu Asn Gln Ser Ser Phe Thr Ala Thr Val Leu Gly Gly Gly
 340 345 350

Ala Gln Glu His Asn Lys Ile Ile Thr Lys Asp Phe Asp Glu Ile Arg
 355 360 365

Asn Ile Ile Lys Asn Asn Ser Val Tyr Ser Pro Gln Asn Pro Gly Tyr
 370 375 380

Pro Ile Ser Tyr Thr Thr Thr Phe Leu Lys Asp Asn Ser Ile Ala Ser
 385 390 395 400

Val Asn Asn Lys Thr Glu Tyr Ile Glu Thr Thr Ala Thr Glu Tyr Thr
 405 410 415

Asn Gly Lys Ile Val Leu Asp His Ser Gly Ala Tyr Val Ala Gln Phe
 420 425 430

Gln Val Thr Trp Asp Glu Val Ser Tyr Asp Glu Lys Gly Asn Glu Ile
 435 440 445

Val Glu His Lys Ala Trp Glu Gly Asn Asn Arg Asp Arg Thr Ala His
 450 455 460

Phe Asn Thr Glu Ile Tyr Leu Lys Gly Asn Ala Arg Asn Ile Ser Val
 465 470 475 480

Lys Ile Arg Glu Cys Thr Gly Leu Ala Trp Glu Trp Trp Arg Thr Ile
 485 490 495

Val Asp Val Lys Asn Ile Pro Leu Ala Lys Glu Arg Thr Phe Tyr Ile
 500 505 510

Trp Gly Thr Thr Leu Tyr Pro Lys Thr Ser Ile Glu Thr Lys Met
 515 520 525

<210> 34

<211> 6129

<212> DNA

<213> Cystic Fibrosis Transmembrane Conductance Regulator (CFTR)

<220>

<221> CDS

<222> (133)..(4575)

<223>

<400> 34

aattggaagc aaatgacatc acagcagggtc agagaaaaag ggttgagcgg caggcaccaca 60
gagtagtagg tctttggcat taggagcttg agcccagacg gccctagcag ggaccccagc 120
gcccgagaga cc atg cag agg tcg cct ctg gaa aag gcc agc gtt gtc tcc 171
Met Gln Arg Ser Pro Leu Glu Lys Ala Ser Val Val Ser
1 5 10
aaa ctt ttt ttc agc tgg acc aga cca att ttg agg aaa gga tac aga 219
Lys Leu Phe Phe Ser Trp Thr Arg Pro Ile Leu Arg Lys Gly Tyr Arg
15 20 25
cag cgc ctg gaa ttg tca gac ata tac caa atc cct tct gtt gat tct 267
Gln Arg Leu Glu Leu Ser Asp Ile Tyr Gln Ile Pro Ser Val Asp Ser
30 35 40 45
gct gac aat cta tct gaa aaa ttg gaa aga gaa tgg gat aga gag ctg 315
Ala Asp Asn Leu Ser Glu Lys Leu Glu Arg Glu Trp Asp Arg Glu Leu
50 55 60
gct tca aag aaa aat cct aaa ctc att aat gcc ctt cgg cga tgt ttt 363
Ala Ser Lys Lys Asn Pro Lys Leu Ile Asn Ala Leu Arg Arg Cys Phe
65 70 75
ttc tgg aga ttt atg ttc tat gga atc ttt tta tat tta ggg gaa gtc 411
Phe Trp Arg Phe Met Phe Tyr Gly Ile Phe Leu Tyr Leu Gly Glu Val
80 85 90
acc aaa gca gta cag cct ctc tta ctg gga aga atc ata gct tcc tat 459
Thr Lys Ala Val Gln Pro Leu Leu Leu Gly Arg Ile Ile Ala Ser Tyr
95 100 105
gac ccg gat aac aag gag gaa cgc tct atc gcg att tat cta ggc ata 507
Asp Pro Asp Asn Lys Glu Glu Arg Ser Ile Ala Ile Tyr Leu Gly Ile
110 115 120 125
ggc tta tgc ctt ctc ttt att gtg agg aca ctg ctc cta cac cca gcc 555
Gly Leu Cys Leu Leu Phe Ile Val Arg Thr Leu Leu Leu His Pro Ala
130 135 140
att ttt ggc ctt cat cac att gga atg cag atg aga ata gct atg ttt 603
Ile Phe Gly Leu His His Ile Gly Met Gln Met Arg Ile Ala Met Phe
145 150 155
agt ttg att tat aag aag act tta aag ctg tca agc cgt gtt cta gat 651
Ser Leu Ile Tyr Lys Lys Thr Leu Lys Leu Ser Ser Arg Val Leu Asp
160 165 170
aaa ata agt att gga caa ctt gtt agt ctc ctt tcc aac aac ctg aac 699
Lys Ile Ser Ile Gly Gln Leu Val Ser Leu Leu Ser Asn Asn Leu Asn
175 180 185
aaa ttt gat gaa gga ctt gca ttg gca cat ttc gtg tgg atc gct cct 747
Lys Phe Asp Glu Gly Leu Ala Leu Ala His Phe Val Trp Ile Ala Pro
190 195 200 205
ttg caa gtg gca ctc ctc atg ggg cta atc tgg gag ttg tta cag gcg 795
Leu Gln Val Ala Leu Leu Met Gly Leu Ile Trp Glu Leu Leu Gln Ala
210 215 220
tct gcc ttc tgt gga ctt ggt ttc ctg ata gtc ctt gcc ctt ttt cag 843
Ser Ala Phe Cys Gly Leu Gly Phe Leu Ile Val Leu Ala Leu Phe Gln
225 230 235

gct ggg cta ggg aga atg atg atg aag tac aga gat cag aga gct ggg Ala Gly Leu Gly Arg Met Met Met Lys Tyr Arg Asp Gln Arg Ala Gly 240 245 250	891
aag atc agt gaa aga ctt gtg att acc tca gaa atg att gaa aat atc Lys Ile Ser Glu Arg Leu Val Ile Thr Ser Glu Met Ile Glu Asn Ile 255 260 265	939
caa tct gtt aag gca tac tgc tgg gaa gaa gca atg gaa aaa atg att Gln Ser Val Lys Ala Tyr Cys Trp Glu Glu Ala Met Glu Lys Met Ile 270 275 280 285	987
gaa aac tta aga caa aca gaa ctg aaa ctg act cgg aag gca gcc tat Glu Asn Leu Arg Gln Thr Glu Leu Lys Leu Thr Arg Lys Ala Ala Tyr 290 295 300	1035
gtg aga tac ttc aat agc tca gcc ttc ttc ttc tca ggg ttc ttt gtg Val Arg Tyr Phe Asn Ser Ser Ala Phe Phe Phe Ser Gly Phe Phe Val 305 310 315	1083
gtg ttt tta tct gtg ctt ccc tat gca cta atc aaa gga atc atc ctc Val Phe Leu Ser Val Leu Pro Tyr Ala Leu Ile Lys Gly Ile Ile Leu 320 325 330	1131
cgg aaa ata ttc acc acc atc tca ttc tgc att gtt ctg cgc atg gcg Arg Lys Ile Phe Thr Thr Ile Ser Phe Cys Ile Val Leu Arg Met Ala 335 340 345	1179
gtc act cgg caa ttt ccc tgg gct gta caa aca tgg tat gac tct ctt Val Thr Arg Gln Phe Pro Trp Ala Val Gln Thr Trp Tyr Asp Ser Leu 350 355 360 365	1227
gga gca ata aac aaa ata cag gat ttc tta caa aag caa gaa tat aag Gly Ala Ile Asn Lys Ile Gln Asp Phe Leu Gln Lys Gln Glu Tyr Lys 370 375 380	1275
aca ttg gaa tat aac tta acg act aca gaa gta gtg atg gag aat gta Thr Leu Glu Tyr Asn Leu Thr Thr Thr Glu Val Val Met Glu Asn Val 385 390 395	1323
aca gcc ttc tgg gag gag gga ttt ggg gaa tta ttt gag aaa gca aaa Thr Ala Phe Trp Glu Glu Gly Phe Gly Glu Leu Phe Glu Lys Ala Lys 400 405 410	1371
caa aac aat aac aat aga aaa act tct aat ggt gat gac agc ctc ttc Gln Asn Asn Asn Asn Arg Lys Thr Ser Asn Gly Asp Asp Ser Leu Phe 415 420 425	1419
ttc agt aat ttc tca ctt ctt ggt act cct gtc ctg aaa gat att aat Phe Ser Asn Phe Ser Leu Leu Gly Thr Pro Val Leu Lys Asp Ile Asn 430 435 440 445	1467
ttc aag ata gaa aga gga cag ttg ttg gcg gtt gct gga tcc act gga Phe Lys Ile Glu Arg Gly Gln Leu Leu Ala Val Ala Gly Ser Thr Gly 450 455 460	1515
gca ggc aag act tca ctt cta atg atg att atg gga gaa ctg gag cct Ala Gly Lys Thr Ser Leu Leu Met Met Ile Met Gly Glu Leu Glu Pro 465 470 475	1563
tca gag ggt aaa att aag cac agt gga aga att tca ttc tgt tct cag Ser Glu Gly Lys Ile Lys His Ser Gly Arg Ile Ser Phe Cys Ser Gln	1611

480	485	490	
ttt tcc tgg att atg cct ggc acc att aaa gaa aat atc atc ttt ggt Phe Ser Trp Ile Met Pro Gly Thr Ile Lys Glu Asn Ile Ile Phe Gly 495 500 505			1659
ggt tcc tat gat gaa tat aga tac aga agc gtc atc aaa gca tgc caa Val Ser Tyr Asp Glu Tyr Arg Tyr Arg Ser Val Ile Lys Ala Cys Gln 510 515 520 525			1707
cta gaa gag gac atc tcc aag ttt gca gag aaa gac aat ata gtt ctt Leu Glu Glu Asp Ile Ser Lys Phe Ala Glu Lys Asp Asn Ile Val Leu 530 535 540			1755
gga gaa ggt gga atc aca ctg agt gga ggt caa cga gca aga att tct Gly Glu Gly Gly Ile Thr Leu Ser Gly Gly Gln Arg Ala Arg Ile Ser 545 550 555			1803
tta gca aga gca gta tac aaa gat gct gat ttg tat tta tta gac tct Leu Ala Arg Ala Val Tyr Lys Asp Ala Asp Leu Tyr Leu Leu Asp Ser 560 565 570			1851
cct ttt gga tac cta gat gtt tta aca gaa aaa gaa ata ttt gaa agc Pro Phe Gly Tyr Leu Asp Val Leu Thr Glu Lys Glu Ile Phe Glu Ser 575 580 585			1899
tgt gtc tgt aaa ctg atg gct aac aaa act agg att ttg gtc act tct Cys Val Cys Lys Leu Met Ala Asn Lys Thr Arg Ile Leu Val Thr Ser 590 595 600 605			1947
aaa atg gaa cat tta aag aaa gct gac aaa ata tta att ttg aat gaa Lys Met Glu His Leu Lys Lys Ala Asp Lys Ile Leu Ile Leu Asn Glu 610 615 620			1995
ggt agc agc tat ttt tat ggg aca ttt tca gaa ctc caa aat cta cag Gly Ser Ser Tyr Phe Tyr Gly Thr Phe Ser Glu Leu Gln Asn Leu Gln 625 630 635			2043
cca gac ttt agc tca aaa ctc atg gga tgt gat tct ttc gac caa ttt Pro Asp Phe Ser Ser Lys Leu Met Gly Cys Asp Ser Phe Asp Gln Phe 640 645 650			2091
agt gca gaa aga aga aat tca atc cta act gag acc tta cac cgt ttc Ser Ala Glu Arg Arg Asn Ser Ile Leu Thr Glu Thr Leu His Arg Phe 655 660 665			2139
tca tta gaa gga gat gct cct gtc tcc tgg aca gaa aca aaa aaa caa Ser Leu Glu Gly Asp Ala Pro Val Ser Trp Thr Glu Thr Lys Lys Gln 670 675 680 685			2187
tct ttt aaa cag act gga gag ttt ggg gaa aaa agg aag aat tct att Ser Phe Lys Gln Thr Gly Glu Phe Gly Glu Lys Arg Lys Asn Ser Ile 690 695 700			2235
ctc aat cca atc aac tct ata cga aaa ttt tcc att gtg caa aag act Leu Asn Pro Ile Asn Ser Ile Arg Lys Phe Ser Ile Val Gln Lys Thr 705 710 715			2283
ccc tta caa atg aat ggc atc gaa gag gat tct gat gag cct tta gag Pro Leu Gln Met Asn Gly Ile Glu Glu Asp Ser Asp Glu Pro Leu Glu 720 725 730			2331
aga agg ctg tcc tta gta cca gat tct gag cag gga gag gcg ata ctg			2379

Arg	Arg	Leu	Ser	Leu	Val	Pro	Asp	Ser	Glu	Gln	Gly	Glu	Ala	Ile	Leu	
735						740					745					
cct	cgc	atc	agc	gtg	atc	agc	act	ggc	ccc	acg	ctt	cag	gca	cga	agg	2427
Pro	Arg	Ile	Ser	Val	Ile	Ser	Thr	Gly	Pro	Thr	Leu	Gln	Ala	Arg	Arg	
750					755					760					765	
agg	cag	tct	gtc	ctg	aac	ctg	atg	aca	cac	tca	gtt	aac	caa	ggc	cag	2475
Arg	Gln	Ser	Val	Leu	Asn	Leu	Met	Thr	His	Ser	Val	Asn	Gln	Gly	Gln	
				770					775					780		
aac	att	cac	cga	aag	aca	aca	gca	tcc	aca	cga	aaa	gtg	tca	ctg	gcc	2523
Asn	Ile	His	Arg	Lys	Thr	Thr	Ala	Ser	Thr	Arg	Lys	Val	Ser	Leu	Ala	
			785					790					795			
cct	cag	gca	aac	ttg	act	gaa	ctg	gat	ata	tat	tca	aga	agg	tta	tct	2571
Pro	Gln	Ala	Asn	Leu	Thr	Glu	Leu	Asp	Ile	Tyr	Ser	Arg	Arg	Leu	Ser	
		800					805					810				
caa	gaa	act	ggc	ttg	gaa	ata	agt	gaa	gaa	att	aac	gaa	gaa	gac	tta	2619
Gln	Glu	Thr	Gly	Leu	Glu	Ile	Ser	Glu	Glu	Ile	Asn	Glu	Glu	Asp	Leu	
		815				820					825					
aag	gag	tgc	ctt	ttt	gat	gat	atg	gag	agc	ata	cca	gca	gtg	act	aca	2667
Lys	Glu	Cys	Leu	Phe	Asp	Asp	Met	Glu	Ser	Ile	Pro	Ala	Val	Thr	Thr	
830					835					840					845	
tgg	aac	aca	tac	ctt	cga	tat	att	act	gtc	cac	aag	agc	tta	att	ttt	2715
Trp	Asn	Thr	Tyr	Leu	Arg	Tyr	Ile	Thr	Val	His	Lys	Ser	Leu	Ile	Phe	
				850					855					860		
gtg	cta	att	tgg	tgc	tta	gta	att	ttt	ctg	gca	gag	gtg	gct	gct	tct	2763
Val	Leu	Ile	Trp	Cys	Leu	Val	Ile	Phe	Leu	Ala	Glu	Val	Ala	Ala	Ser	
			865					870					875			
ttg	gtt	gtg	ctg	tgg	ctc	ctt	gga	aac	act	cct	ctt	caa	gac	aaa	ggg	2811
Leu	Val	Val	Leu	Trp	Leu	Leu	Gly	Asn	Thr	Pro	Leu	Gln	Asp	Lys	Gly	
			880				885					890				
aat	agt	act	cat	agt	aga	aat	aac	agc	tat	gca	gtg	att	atc	acc	agc	2859
Asn	Ser	Thr	His	Ser	Arg	Asn	Asn	Ser	Tyr	Ala	Val	Ile	Ile	Thr	Ser	
			895			900					905					
acc	agt	tcg	tat	tat	gtg	ttt	tac	att	tac	gtg	gga	gta	gcc	gac	act	2907
Thr	Ser	Ser	Tyr	Tyr	Val	Phe	Tyr	Ile	Tyr	Val	Gly	Val	Ala	Asp	Thr	
910					915					920					925	
ttg	ctt	gct	atg	gga	ttc	ttc	aga	ggc	cta	cca	ctg	gtg	cat	act	cta	2955
Leu	Leu	Ala	Met	Gly	Phe	Phe	Arg	Gly	Leu	Pro	Leu	Val	His	Thr	Leu	
				930					935					940		
atc	aca	gtg	tcg	aaa	att	tta	cac	cac	aaa	atg	tta	cat	tct	gtt	ctt	3003
Ile	Thr	Val	Ser	Lys	Ile	Leu	His	His	Lys	Met	Leu	His	Ser	Val	Leu	
				945				950						955		
caa	gca	cct	atg	tca	acc	ctc	aac	acg	ttg	aaa	gca	ggc	ggg	att	ctt	3051
Gln	Ala	Pro	Met	Ser	Thr	Leu	Asn	Thr	Leu	Lys	Ala	Gly	Gly	Ile	Leu	
			960				965					970				
aat	aga	ttc	tcc	aaa	gat	ata	gca	att	ttg	gat	gac	ctt	ctg	cct	ctt	3099
Asn	Arg	Phe	Ser	Lys	Asp	Ile	Ala	Ile	Leu	Asp	Asp	Leu	Leu	Pro	Leu	
				975		980					985					

acc ata ttt gac ttc atc cag ttg tta tta att	gtg att gga gct ata	3147
Thr Ile Phe Asp Phe Ile Gln Leu Leu Leu Ile	Val Ile Gly Ala Ile	
990	995 1000 1005	
gca gtt gtc gca gtt tta caa ccc tac atc	ttt gtt gca aca gtg	3192
Ala Val Val Ala Val Leu Gln Pro Tyr Ile	Phe Val Ala Thr Val	
1010	1015 1020	
cca gtg ata gtg gct ttt att atg ttg aga	gca tat ttc ctc caa	3237
Pro Val Ile Val Ala Phe Ile Met Leu Arg	Ala Tyr Phe Leu Gln	
1025	1030 1035	
acc tca cag caa ctc aaa caa ctg gaa tct	gaa ggc agg agt cca	3282
Thr Ser Gln Gln Leu Lys Gln Leu Glu Ser	Glu Gly Arg Ser Pro	
1040	1045 1050	
att ttc act cat ctt gtt aca agc tta aaa	gga cta tgg aca ctt	3327
Ile Phe Thr His Leu Val Thr Ser Leu Lys	Gly Leu Trp Thr Leu	
1055	1060 1065	
cgt gcc ttc gga cgg cag cct tac ttt gaa	act ctg ttc cac aaa	3372
Arg Ala Phe Gly Arg Gln Pro Tyr Phe Glu	Thr Leu Phe His Lys	
1070	1075 1080	
gct ctg aat tta cat act gcc aac tgg ttc	ttg tac ctg tca aca	3417
Ala Leu Asn Leu His Thr Ala Asn Trp Phe	Leu Tyr Leu Ser Thr	
1085	1090 1095	
ctg cgc tgg ttc caa atg aga ata gaa atg	att ttt gtc atc ttc	3462
Leu Arg Trp Phe Gln Met Arg Ile Glu Met	Ile Phe Val Ile Phe	
1100	1105 1110	
ttc att gct gtt acc ttc att tcc att tta	aca aca gga gaa gga	3507
Phe Ile Ala Val Thr Phe Ile Ser Ile Leu	Thr Thr Gly Glu Gly	
1115	1120 1125	
gaa gga aga gtt ggt att atc ctg act tta	gcc atg aat atc atg	3552
Glu Gly Arg Val Gly Ile Ile Leu Thr Leu	Ala Met Asn Ile Met	
1130	1135 1140	
agt aca ttg cag tgg gct gta aac tcc agc	ata gat gtg gat agc	3597
Ser Thr Leu Gln Trp Ala Val Asn Ser Ser	Ile Asp Val Asp Ser	
1145	1150 1155	
ttg atg cga tct gtg agc cga gtc ttt aag	ttc att gac atg cca	3642
Leu Met Arg Ser Val Ser Arg Val Phe Lys	Phe Ile Asp Met Pro	
1160	1165 1170	
aca gaa ggt aaa cct acc aag tca acc aaa	cca tac aag aat ggc	3687
Thr Glu Gly Lys Pro Thr Lys Ser Thr Lys	Pro Tyr Lys Asn Gly	
1175	1180 1185	
caa ctc tcg aaa gtt atg att att gag aat	tca cac gtg aag aaa	3732
Gln Leu Ser Lys Val Met Ile Ile Glu Asn	Ser His Val Lys Lys	
1190	1195 1200	
gat gac atc tgg ccc tca ggg ggc caa atg	act gtc aaa gat ctc	3777
Asp Asp Ile Trp Pro Ser Gly Gly Gln Met	Thr Val Lys Asp Leu	
1205	1210 1215	
aca gca aaa tac aca gaa ggt gga aat gcc	ata tta gag aac att	3822
Thr Ala Lys Tyr Thr Glu Gly Gly Asn Ala	Ile Leu Glu Asn Ile	
1220	1225 1230	

tcc ttc tca ata agt cct ggc cag agg gtg ggc ctc ttg gga aga Ser Phe Ser Ile Ser Pro Gly Gln Arg Val Gly Leu Leu Gly Arg 1235 1240 1245	3867
act gga tca ggg aag agt act ttg tta tca gct ttt ttg aga cta Thr Gly Ser Gly Lys Ser Thr Leu Leu Ser Ala Phe Leu Arg Leu 1250 1255 1260	3912
ctg aac act gaa gga gaa atc cag atc gat ggt gtg tct tgg gat Leu Asn Thr Glu Gly Glu Ile Gln Ile Asp Gly Val Ser Trp Asp 1265 1270 1275	3957
tca ata act ttg caa cag tgg agg aaa gcc ttt gga gtg ata cca Ser Ile Thr Leu Gln Gln Trp Arg Lys Ala Phe Gly Val Ile Pro 1280 1285 1290	4002
cag aaa gta ttt att ttt tct gga aca ttt aga aaa aac ttg gat Gln Lys Val Phe Ile Phe Ser Gly Thr Phe Arg Lys Asn Leu Asp 1295 1300 1305	4047
ccc tat gaa cag tgg agt gat caa gaa ata tgg aaa gtt gca gat Pro Tyr Glu Gln Trp Ser Asp Gln Glu Ile Trp Lys Val Ala Asp 1310 1315 1320	4092
gag gtt ggg ctc aga tct gtg ata gaa cag ttt cct ggg aag ctt Glu Val Gly Leu Arg Ser Val Ile Glu Gln Phe Pro Gly Lys Leu 1325 1330 1335	4137
gac ttt gtc ctt gtg gat ggg ggc tgt gtc cta agc cat ggc cac Asp Phe Val Leu Val Asp Gly Gly Cys Val Leu Ser His Gly His 1340 1345 1350	4182
aag cag ttg atg tgc ttg gct aga tct gtt ctc agt aag gcg aag Lys Gln Leu Met Cys Leu Ala Arg Ser Val Leu Ser Lys Ala Lys 1355 1360 1365	4227
atc ttg ctg ctt gat gaa ccc agt gct cat ttg gat cca gta aca Ile Leu Leu Leu Asp Glu Pro Ser Ala His Leu Asp Pro Val Thr 1370 1375 1380	4272
tac caa ata att aga aga act cta aaa caa gca ttt gct gat tgc Tyr Gln Ile Ile Arg Arg Thr Leu Lys Gln Ala Phe Ala Asp Cys 1385 1390 1395	4317
aca gta att ctc tgt gaa cac agg ata gaa gca atg ctg gaa tgc Thr Val Ile Leu Cys Glu His Arg Ile Glu Ala Met Leu Glu Cys 1400 1405 1410	4362
caa caa ttt ttg gtc ata gaa gag aac aaa gtg cgg cag tac gat Gln Gln Phe Leu Val Ile Glu Glu Asn Lys Val Arg Gln Tyr Asp 1415 1420 1425	4407
tcc atc cag aaa ctg ctg aac gag agg agc ctc ttc cgg caa gcc Ser Ile Gln Lys Leu Leu Asn Glu Arg Ser Leu Phe Arg Gln Ala 1430 1435 1440	4452
atc agc ccc tcc gac agg gtg aag ctc ttt ccc cac cgg aac tca Ile Ser Pro Ser Asp Arg Val Lys Leu Phe Pro His Arg Asn Ser 1445 1450 1455	4497
agc aag tgc aag tct aag ccc cag att gct gct ctg aaa gag gag Ser Lys Cys Lys Ser Lys Pro Gln Ile Ala Ala Leu Lys Glu Glu 1455 1460 1465	4542

1460	1465	1470	
aca gaa gaa gag gtg caa gat aca agg ctt tag agagcagcat			4585
Thr Glu Glu Glu Val Gln Asp Thr Arg Leu			
1475	1480		
aaatgttgac atgggacatt tgctcatgga attggagctc gtgggacagt cacctcatgg			4645
aattggagct cgtggaacag ttacctctgc ctcagaaaac aaggatgaat taagtttttt			4705
tttaaaaaag aaacatttgg taaggggaat tgaggacact gatatgggtc ttgataaatg			4765
gcttcctggc aatagtcaaa ttgtgtgaaa ggtacttcaa atccttgaag atttaccact			4825
tgtgttttgc aagccagatt ttcctgaaaa cccttgccat gtgctagtaa ttggaaaggc			4885
agctctaaat gtcaatcagc ctagttagtc agcttattgt ctagtgaac tcgttaattt			4945
gtagtgttgg agaagaactg aaatcatact tcttaggggt atgattaagt aatgataact			5005
ggaaacttca gcggtttata taagcttgta ttcctttttc tctcctctcc ccatgatgtt			5065
tagaaacaca actatattgt ttgctaagca ttccaactat ctcatttcca agcaagtatt			5125
agaataccac aggaaccaca agactgcaca tcaaaatatg cccatttcaa catctagtga			5185
gcagtcagga aagagaactt ccagatcctg gaaatcaggg ttagtattgt ccaggtctac			5245
caaaaatctc aatatttcag ataatcacaa tacatccctt acctgggaaa gggctgttat			5305
aatctttcac aggggacagg atggttccct tgatgaagaa gttgatatgc cttttcccaa			5365
ctccagaaaag tgacaagctc acagaccttt gaactagagt ttagctggaa aagtatgtta			5425
gtgcaaattg tcacaggaca gcccttcttt ccacagaagc tccaggtaga ggggtgttaa			5485
gtagataggc catgggcact gtgggtagac acacatgaag tccaagcatt tagatgtata			5545
ggttgatggg ggtatgtttt caggctagat gtatgtactt catgctgtct aactaagag			5605
agaatgagag acacactgaa gaagcaccaa tcatgaatta gttttatatg cttctgtttt			5665
ataattttgt gaagcaaaat tttttctcta ggaaatatat attttaataa tgtttcaaac			5725
atatattaca atgctgtatt ttaaaagaat gattatgaat tacatttgta taaaataatt			5785
tttatatttg aaatattgac tttttatggc actagtattt ttatgaaata ttatgttaaa			5845
actgggacag gggagaacct agggtagatat taaccagggg ccatgaatca ccttttggtc			5905
tggagggaag ccttggggct gatcgagttg ttgccacag ctgtatgatt cccagccaga			5965
cacagcctct tagatgcagt tctgaagaag atggtaccac cagtctgact gtttccatca			6025
agggtagact gccttctcaa ctccaaactg actcttaaga agactgcatt atatttatta			6085
ctgtaagaaa atatcacttg tcaataaaaat ccatacatth gtgt			6129
<210> 35			
<211> 1480			
<212> PRT			
<213> Cystic Fibrosis Transmembrane Conductance Regulator (CFTR)			

<400> 35

Met Gln Arg Ser Pro Leu Glu Lys Ala Ser Val Val Ser Lys Leu Phe
 1 5 10 15

Phe Ser Trp Thr Arg Pro Ile Leu Arg Lys Gly Tyr Arg Gln Arg Leu
 20 25 30

Glu Leu Ser Asp Ile Tyr Gln Ile Pro Ser Val Asp Ser Ala Asp Asn
 35 40 45

Leu Ser Glu Lys Leu Glu Arg Glu Trp Asp Arg Glu Leu Ala Ser Lys
 50 55 60

Lys Asn Pro Lys Leu Ile Asn Ala Leu Arg Arg Cys Phe Phe Trp Arg
 65 70 75 80

Phe Met Phe Tyr Gly Ile Phe Leu Tyr Leu Gly Glu Val Thr Lys Ala
 85 90 95

Val Gln Pro Leu Leu Leu Gly Arg Ile Ile Ala Ser Tyr Asp Pro Asp
 100 105 110

Asn Lys Glu Glu Arg Ser Ile Ala Ile Tyr Leu Gly Ile Gly Leu Cys
 115 120 125

Leu Leu Phe Ile Val Arg Thr Leu Leu Leu His Pro Ala Ile Phe Gly
 130 135 140

Leu His His Ile Gly Met Gln Met Arg Ile Ala Met Phe Ser Leu Ile
 145 150 155 160

Tyr Lys Lys Thr Leu Lys Leu Ser Ser Arg Val Leu Asp Lys Ile Ser
 165 170 175

Ile Gly Gln Leu Val Ser Leu Leu Ser Asn Asn Leu Asn Lys Phe Asp
 180 185 190

Glu Gly Leu Ala Leu Ala His Phe Val Trp Ile Ala Pro Leu Gln Val
 195 200 205

Ala Leu Leu Met Gly Leu Ile Trp Glu Leu Leu Gln Ala Ser Ala Phe
 210 215 220

Cys Gly Leu Gly Phe Leu Ile Val Leu Ala Leu Phe Gln Ala Gly Leu
 225 230 235 240

Gly Arg Met Met Met Lys Tyr Arg Asp Gln Arg Ala Gly Lys Ile Ser
 245 250 255

Glu Arg Leu Val Ile Thr Ser Glu Met Ile Glu Asn Ile Gln Ser Val
 260 265 270

Lys Ala Tyr Cys Trp Glu Glu Ala Met Glu Lys Met Ile Glu Asn Leu
 275 280 285

Arg Gln Thr Glu Leu Lys Leu Thr Arg Lys Ala Ala Tyr Val Arg Tyr
 290 295 300

Phe Asn Ser Ser Ala Phe Phe Phe Ser Gly Phe Phe Val Val Phe Leu
 305 310 315 320

Ser Val Leu Pro Tyr Ala Leu Ile Lys Gly Ile Ile Leu Arg Lys Ile
 325 330 335

Phe Thr Thr Ile Ser Phe Cys Ile Val Leu Arg Met Ala Val Thr Arg
 340 345 350

Gln Phe Pro Trp Ala Val Gln Thr Trp Tyr Asp Ser Leu Gly Ala Ile
 355 360 365

Asn Lys Ile Gln Asp Phe Leu Gln Lys Gln Glu Tyr Lys Thr Leu Glu
 370 375 380

Tyr Asn Leu Thr Thr Thr Glu Val Val Met Glu Asn Val Thr Ala Phe
 385 390 395 400

Trp Glu Glu Gly Phe Gly Glu Leu Phe Glu Lys Ala Lys Gln Asn Asn
 405 410 415

Asn Asn Arg Lys Thr Ser Asn Gly Asp Asp Ser Leu Phe Phe Ser Asn
 420 425 430

Phe Ser Leu Leu Gly Thr Pro Val Leu Lys Asp Ile Asn Phe Lys Ile
 435 440 445

Glu Arg Gly Gln Leu Leu Ala Val Ala Gly Ser Thr Gly Ala Gly Lys
 450 455 460

Thr Ser Leu Leu Met Met Ile Met Gly Glu Leu Glu Pro Ser Glu Gly
 465 470 475 480

Lys Ile Lys His Ser Gly Arg Ile Ser Phe Cys Ser Gln Phe Ser Trp
 485 490 495

Ile Met Pro Gly Thr Ile Lys Glu Asn Ile Ile Phe Gly Val Ser Tyr
 500 505 510

Asp Glu Tyr Arg Tyr Arg Ser Val Ile Lys Ala Cys Gln Leu Glu Glu
 515 520 525

Asp Ile Ser Lys Phe Ala Glu Lys Asp Asn Ile Val Leu Gly Glu Gly
 530 535 540

Gly Ile Thr Leu Ser Gly Gly Gln Arg Ala Arg Ile Ser Leu Ala Arg
 545 550 555 560

Ala Val Tyr Lys Asp Ala Asp Leu Tyr Leu Leu Asp Ser Pro Phe Gly
 565 570 575

Tyr Leu Asp Val Leu Thr Glu Lys Glu Ile Phe Glu Ser Cys Val Cys
 580 585 590

Lys Leu Met Ala Asn Lys Thr Arg Ile Leu Val Thr Ser Lys Met Glu
 595 600 605

His Leu Lys Lys Ala Asp Lys Ile Leu Ile Leu Asn Glu Gly Ser Ser
 610 615 620

Tyr Phe Tyr Gly Thr Phe Ser Glu Leu Gln Asn Leu Gln Pro Asp Phe
 625 630 635 640

Ser Ser Lys Leu Met Gly Cys Asp Ser Phe Asp Gln Phe Ser Ala Glu
 645 650 655

Arg Arg Asn Ser Ile Leu Thr Glu Thr Leu His Arg Phe Ser Leu Glu
 660 665 670

Gly Asp Ala Pro Val Ser Trp Thr Glu Thr Lys Lys Gln Ser Phe Lys
 675 680 685

Gln Thr Gly Glu Phe Gly Glu Lys Arg Lys Asn Ser Ile Leu Asn Pro
 690 695 700

Ile Asn Ser Ile Arg Lys Phe Ser Ile Val Gln Lys Thr Pro Leu Gln
 705 710 715 720

Met Asn Gly Ile Glu Glu Asp Ser Asp Glu Pro Leu Glu Arg Arg Leu
 725 730 735

Ser Leu Val Pro Asp Ser Glu Gln Gly Glu Ala Ile Leu Pro Arg Ile
 740 745 750

Ser Val Ile Ser Thr Gly Pro Thr Leu Gln Ala Arg Arg Arg Gln Ser
 755 760 765

Val Leu Asn Leu Met Thr His Ser Val Asn Gln Gly Gln Asn Ile His
 770 775 780

Arg Lys Thr Thr Ala Ser Thr Arg Lys Val Ser Leu Ala Pro Gln Ala
 785 790 795 800

Asn Leu Thr Glu Leu Asp Ile Tyr Ser Arg Arg Leu Ser Gln Glu Thr
 805 810 815

Gly Leu Glu Ile Ser Glu Glu Ile Asn Glu Glu Asp Leu Lys Glu Cys
 820 825 830

Leu Phe Asp Asp Met Glu Ser Ile Pro Ala Val Thr Thr Trp Asn Thr
 835 840 845

Tyr Leu Arg Tyr Ile Thr Val His Lys Ser Leu Ile Phe Val Leu Ile
 850 855 860

Trp Cys Leu Val Ile Phe Leu Ala Glu Val Ala Ala Ser Leu Val Val
 865 870 875 880

Leu Trp Leu Leu Gly Asn Thr Pro Leu Gln Asp Lys Gly Asn Ser Thr
 885 890 895

His Ser Arg Asn Asn Ser Tyr Ala Val Ile Ile Thr Ser Thr Ser Ser
 900 905 910

Tyr Tyr Val Phe Tyr Ile Tyr Val Gly Val Ala Asp Thr Leu Leu Ala
 915 920 925

Met Gly Phe Phe Arg Gly Leu Pro Leu Val His Thr Leu Ile Thr Val
 930 935 940

Ser Lys Ile Leu His His Lys Met Leu His Ser Val Leu Gln Ala Pro
 945 950 955 960

Met Ser Thr Leu Asn Thr Leu Lys Ala Gly Gly Ile Leu Asn Arg Phe
 965 970 975

Ser Lys Asp Ile Ala Ile Leu Asp Asp Leu Leu Pro Leu Thr Ile Phe
 980 985 990

Asp Phe Ile Gln Leu Leu Leu Ile Val Ile Gly Ala Ile Ala Val Val

995	1000	1005
Ala Val 1010	Leu Gln Pro Tyr Ile 1015	Phe Val Ala Thr Val 1020
Val Ala 1025	Phe Ile Met Leu Arg 1030	Ala Tyr Phe Leu Gln 1035
Gln Leu 1040	Lys Gln Leu Glu Ser 1045	Glu Gly Arg Ser Pro 1050
His Leu 1055	Val Thr Ser Leu Lys 1060	Gly Leu Trp Thr Leu 1065
Gly Arg 1070	Gln Pro Tyr Phe Glu 1075	Thr Leu Phe His Lys 1080
Leu His 1085	Thr Ala Asn Trp Phe 1090	Leu Tyr Leu Ser Thr 1095
Phe Gln 1100	Met Arg Ile Glu Met 1105	Ile Phe Val Ile Phe 1110
Val Thr 1115	Phe Ile Ser Ile Leu 1120	Thr Thr Gly Glu Gly 1125
Val Gly 1130	Ile Ile Leu Thr Leu 1135	Ala Met Asn Ile Met 1140
Gln Trp 1145	Ala Val Asn Ser Ser 1150	Ile Asp Val Asp Ser 1155
Ser Val 1160	Ser Arg Val Phe Lys 1165	Phe Ile Asp Met Pro 1170
Lys Pro 1175	Thr Lys Ser Thr Lys 1180	Pro Tyr Lys Asn Gly 1185
Lys Val 1190	Met Ile Ile Glu Asn 1195	Ser His Val Lys Lys 1200
Trp Pro 1205	Ser Gly Gly Gln Met 1210	Thr Val Lys Asp Leu 1215
Tyr Thr 1220	Glu Gly Gly Asn Ala 1225	Ile Leu Glu Asn Ile 1230

Glu Val Gln Asp Thr Arg Leu
1475 1480

<210> 36
<211> 3168
<212> DNA
<213> GLUT2

<220>
<221> CDS
<222> (39)..(1613)
<223>

<400> 36
cacaagacct ggaattgaca ggactcccaa ctagtaca atg aca gaa gat aag gtc 56
Met Thr Glu Asp Lys Val
1 5

act ggg acc ctg gtt ttc act gtc atc act gct gtg ctg ggt tcc ttc 104
Thr Gly Thr Leu Val Phe Thr Val Ile Thr Ala Val Leu Gly Ser Phe
10 15 20

cag ttt gga tat gac att ggt gtg atc aat gca cct caa cag gta ata 152
Gln Phe Gly Tyr Asp Ile Gly Val Ile Asn Ala Pro Gln Gln Val Ile
25 30 35

ata tct cac tat aga cat gtt ttg ggt gtt cca ctg gat gac cga aaa 200
Ile Ser His Tyr Arg His Val Leu Gly Val Pro Leu Asp Asp Arg Lys
40 45 50

gct atc aac aac tat gtt atc aac agt aca gat gaa ctg ccc aca atc 248
Ala Ile Asn Asn Tyr Val Ile Asn Ser Thr Asp Glu Leu Pro Thr Ile
55 60 65 70

tca tac tca atg aac cca aaa cca acc cct tgg gct gag gaa gag act 296
Ser Tyr Ser Met Asn Pro Lys Pro Thr Pro Trp Ala Glu Glu Glu Thr
75 80 85

gtg gca gct gct caa cta atc acc atg ctc tgg tcc ctg tct gta tcc 344
Val Ala Ala Ala Gln Leu Ile Thr Met Leu Trp Ser Leu Ser Val Ser
90 95 100

agc ttt gca gtt ggt gga atg act gca tca ttc ttt ggt ggg tgg ctt 392
Ser Phe Ala Val Gly Gly Met Thr Ala Ser Phe Phe Gly Gly Trp Leu
105 110 115

ggg gac aca ctt gga aga atc aaa gcc atg tta gta gca aac att ctg 440
Gly Asp Thr Leu Gly Arg Ile Lys Ala Met Leu Val Ala Asn Ile Leu
120 125 130

tca tta gtt gga gct ctc ttg atg ggg ttt tca aaa ttg gga cca tct 488
Ser Leu Val Gly Ala Leu Leu Met Gly Phe Ser Lys Leu Gly Pro Ser
135 140 145 150

cat ata ctt ata att gct gga aga agc ata tca gga cta tat tgt ggg 536
His Ile Leu Ile Ile Ala Gly Arg Ser Ile Ser Gly Leu Tyr Cys Gly
155 160 165

cta att tca ggc ctg gtt cct atg tat atc ggt gaa att gct cca acc 584
Leu Ile Ser Gly Leu Val Pro Met Tyr Ile Gly Glu Ile Ala Pro Thr
170 175 180

gct ctc agg gga gca ctt ggc act ttt cat cag ctg gcc atc gtc acg Ala Leu Arg Gly Ala Leu Gly Thr Phe His Gln Leu Ala Ile Val Thr 185 190 195	632
ggc att ctt att agt cag att att ggt ctt gaa ttt atc ttg ggc aat Gly Ile Leu Ile Ser Gln Ile Ile Gly Leu Glu Phe Ile Leu Gly Asn 200 205 210	680
tat gat ctg tgg cac atc ctg ctt ggc ctg tct ggt gtg cga gcc atc Tyr Asp Leu Trp His Ile Leu Leu Gly Leu Ser Gly Val Arg Ala Ile 215 220 225 230	728
ctt cag tct ctg cta ctc ttt ttc tgt cca gaa agc ccc aga tac ctt Leu Gln Ser Leu Leu Phe Phe Cys Pro Glu Ser Pro Arg Tyr Leu 235 240 245	776
tac atc aag tta gat gag gaa gtc aaa gca aaa caa agc ttg aaa aga Tyr Ile Lys Leu Asp Glu Glu Val Lys Ala Lys Gln Ser Leu Lys Arg 250 255 260	824
ctc aga gga tat gat gat gtc acc aaa gat att aat gaa atg aga aaa Leu Arg Gly Tyr Asp Asp Val Thr Lys Asp Ile Asn Glu Met Arg Lys 265 270 275	872
gaa aga gaa gaa gca tcg agt gag cag aaa gtc tct ata att cag ctc Glu Arg Glu Glu Ala Ser Ser Glu Gln Lys Val Ser Ile Ile Gln Leu 280 285 290	920
ttc acc aat tcc agc tac cga cag cct att cta gtg gca ctg atg ctg Phe Thr Asn Ser Ser Tyr Arg Gln Pro Ile Leu Val Ala Leu Met Leu 295 300 305 310	968
cat gtg gct cag caa ttt tcc gga atc aat ggc att ttt tac tac tca His Val Ala Gln Gln Phe Ser Gly Ile Asn Gly Ile Phe Tyr Tyr Ser 315 320 325	1016
acc agc att ttt cag acg gct ggt atc agc aaa cct gtt tat gca acc Thr Ser Ile Phe Gln Thr Ala Gly Ile Ser Lys Pro Val Tyr Ala Thr 330 335 340	1064
att gga gtt ggc gct gta aac atg gtt ttc act gct gtc tct gta ttc Ile Gly Val Gly Ala Val Asn Met Val Phe Thr Ala Val Ser Val Phe 345 350 355	1112
ctt gtg gag aag gca ggg cga cgt tct ctc ttt cta att gga atg agt Leu Val Glu Lys Ala Gly Arg Arg Ser Leu Phe Leu Ile Gly Met Ser 360 365 370	1160
ggg atg ttt gtt tgt gcc atc ttc atg tca gtg gga ctt gtg ctg ctg Gly Met Phe Val Cys Ala Ile Phe Met Ser Val Gly Leu Val Leu Leu 375 380 385 390	1208
aat aag ttc tct tgg atg agt tat gtg agc atg ata gcc atc ttc ctc Asn Lys Phe Ser Trp Met Ser Tyr Val Ser Met Ile Ala Ile Phe Leu 395 400 405	1256
ttt gtc agc ttc ttt gaa att ggg cca ggc ccg atc ccc tgg ttc atg Phe Val Ser Phe Phe Glu Ile Gly Pro Gly Pro Ile Pro Trp Phe Met 410 415 420	1304
gtg gct gag ttt ttc agt caa gga cca cgt cct gct gct tta gca ata Val Ala Glu Phe Phe Ser Gln Gly Pro Arg Pro Ala Ala Leu Ala Ile 430 435 440	1352

425	430	435	
gct gca ttc agc aat tgg acc tgc aat ttc att gta gct ctg tgt ttc			1400
Ala Ala Phe Ser Asn Trp Thr Cys Asn Phe Ile Val Ala Leu Cys Phe			
440	445	450	
cag tac att gcg gac ttc tgt gga cct tat gtg ttt ttc ctg ttt gct			1448
Gln Tyr Ile Ala Asp Phe Cys Gly Pro Tyr Val Phe Phe Leu Phe Ala			
455	460	465	470
gga gtg ctg ctg gcc ttt acc ctg ttc aca ttt ttt aaa gtt cca gaa			1496
Gly Val Leu Leu Ala Phe Thr Leu Phe Thr Phe Phe Lys Val Pro Glu			
	475	480	485
acc aaa gga aag tct ttt gag gaa att gct gca gaa ttc caa aag aag			1544
Thr Lys Gly Lys Ser Phe Glu Glu Ile Ala Ala Glu Phe Gln Lys Lys			
	490	495	500
agt ggc tca gcc cac agg cca aaa gct gct gta gaa atg aaa ttc cta			1592
Ser Gly Ser Ala His Arg Pro Lys Ala Ala Val Glu Met Lys Phe Leu			
	505	510	515
gga gct aca gag act gtg taa aaaaaaacc ctgctttttg acatgaacag			1643
Gly Ala Thr Glu Thr Val			
520			
aaacaataag ggaaccgtct gtttttaaat gatgattcct tgagcatttt atatccacat			1703
ctttaagtat tgttttattt ttatgtgctc tcatacagaaa tgtcatcaaa tattaccaa			1763
aaagtatttt ttttaagttag agaatatatt tttgatggta agactgtaat taagtaaacc			1823
aaaaaggcta gtttattttg ttactactaa gggcagggtg ttctaataatt tttagctctg			1883
ttctttataa caaggttctt ctaaaattga agagatttca acatatcatt tttttaacac			1943
ataactagaa acctgaggat gcaacaaata tttatatatt tgaatatcat taaattggaa			2003
ttttcttacc catatatctt atgttaaagg agatatggct agtggcaata agttccatgt			2063
taaaatagac aactcttcca tttattgcac tcagcttttt tcttgagtac tagaatttgt			2123
attttgctta aaattttact tttgttctgt attttcatgt ggaatggatt atagagtata			2183
ctaaaaaatg tctatagaga aaaactttca tttttggtag gcttatcaaa atctttcagc			2243
actcagaaaa gaaaaccatt ttagttcctt tatttaattg ccaaatgggt tttgcaagat			2303
ttaacactaa aaaggtttca cctgatcata tagcgtgggt tatcagttaa cattaacatc			2363
tattataaaa ccatgttgat tcccttctgg tacaatcctt tgagttatag tttgctttgc			2423
tttttaattg aggacagcct ggttttcaca tacactcaaa caatcatgag tcagacattt			2483
ggtatattac ctcaaattcc taataagttt gatcaaact aatgtaagaa aatttgaagt			2543
aaaggattga tcactttgtt aaaaatatatt tctgaattat tatgtctcaa aataagttga			2603
aaaggtaggg tttgaggatt cctgagtgtg ggcttctgaa acttcataaa tgttcagctt			2663
cagactttta tcaaaatccc tatttaattt tcctggaaag actgattgtt ttatggtgtg			2723
ttcctaacat aaaataatcg tctcctttga ctttccttc tttgtcttag ctgtatacag			2783

attctagcca aactattcta tggccattac taacacgcat tgtacactat ctatctgcct 2843
 ttacctacat aggcaaattg gaaatacaca gatgattaaa cagacttttag cttacagtca 2903
 attttacaat tatggaaata tagttctgat ggggtcccaa agcttagcag ggtgctaacg 2963
 tatctctagg ctgttttctc caccaactgg agcactgac aatccttctt atgtttgctt 3023
 taatgtgtat tgaagaaaag cactttttaa aaagtactct ttaagagtga aataattaaa 3083
 aaccactgaa catttgcttt gttttctaaa gttgttcaca tatatgtaat ttagcagtc 3143
 aaagaacaag aaattgtttc ttttc 3168

<210> 37
 <211> 524
 <212> PRT
 <213> GLUT2

<400> 37

Met Thr Glu Asp Lys Val Thr Gly Thr Leu Val Phe Thr Val Ile Thr
 1 5 10 15

Ala Val Leu Gly Ser Phe Gln Phe Gly Tyr Asp Ile Gly Val Ile Asn
 20 25 30

Ala Pro Gln Gln Val Ile Ile Ser His Tyr Arg His Val Leu Gly Val
 35 40 45

Pro Leu Asp Asp Arg Lys Ala Ile Asn Asn Tyr Val Ile Asn Ser Thr
 50 55 60

Asp Glu Leu Pro Thr Ile Ser Tyr Ser Met Asn Pro Lys Pro Thr Pro
 65 70 75 80

Trp Ala Glu Glu Glu Thr Val Ala Ala Ala Gln Leu Ile Thr Met Leu
 85 90 95

Trp Ser Leu Ser Val Ser Ser Phe Ala Val Gly Gly Met Thr Ala Ser
 100 105 110

Phe Phe Gly Gly Trp Leu Gly Asp Thr Leu Gly Arg Ile Lys Ala Met
 115 120 125

Leu Val Ala Asn Ile Leu Ser Leu Val Gly Ala Leu Leu Met Gly Phe
 130 135 140

Ser Lys Leu Gly Pro Ser His Ile Leu Ile Ile Ala Gly Arg Ser Ile
 145 150 155 160

Ser Gly Leu Tyr Cys Gly Leu Ile Ser Gly Leu Val Pro Met Tyr Ile
 165 170 175

Gly Glu Ile Ala Pro Thr Ala Leu Arg Gly Ala Leu Gly Thr Phe His
 180 185 190

Gln Leu Ala Ile Val Thr Gly Ile Leu Ile Ser Gln Ile Ile Gly Leu
 195 200 205

Glu Phe Ile Leu Gly Asn Tyr Asp Leu Trp His Ile Leu Leu Gly Leu
 210 215 220

Ser Gly Val Arg Ala Ile Leu Gln Ser Leu Leu Leu Phe Phe Cys Pro
 225 230 235 240

Glu Ser Pro Arg Tyr Leu Tyr Ile Lys Leu Asp Glu Glu Val Lys Ala
 245 250 255

Lys Gln Ser Leu Lys Arg Leu Arg Gly Tyr Asp Asp Val Thr Lys Asp
 260 265 270

Ile Asn Glu Met Arg Lys Glu Arg Glu Glu Ala Ser Ser Glu Gln Lys
 275 280 285

Val Ser Ile Ile Gln Leu Phe Thr Asn Ser Ser Tyr Arg Gln Pro Ile
 290 295 300

Leu Val Ala Leu Met Leu His Val Ala Gln Gln Phe Ser Gly Ile Asn
 305 310 315 320

Gly Ile Phe Tyr Tyr Ser Thr Ser Ile Phe Gln Thr Ala Gly Ile Ser
 325 330 335

Lys Pro Val Tyr Ala Thr Ile Gly Val Gly Ala Val Asn Met Val Phe
 340 345 350

Thr Ala Val Ser Val Phe Leu Val Glu Lys Ala Gly Arg Arg Ser Leu
 355 360 365

Phe Leu Ile Gly Met Ser Gly Met Phe Val Cys Ala Ile Phe Met Ser
 370 375 380

Val Gly Leu Val Leu Leu Asn Lys Phe Ser Trp Met Ser Tyr Val Ser
 385 390 395 400

Met Ile Ala Ile Phe Leu Phe Val Ser Phe Phe Glu Ile Gly Pro Gly
 405 410 415

Pro Ile Pro Trp Phe Met Val Ala Glu Phe Phe Ser Gln Gly Pro Arg
 420 425 430

Pro Ala Ala Leu Ala Ile Ala Ala Phe Ser Asn Trp Thr Cys Asn Phe
 435 440 445

Ile Val Ala Leu Cys Phe Gln Tyr Ile Ala Asp Phe Cys Gly Pro Tyr
 450 455 460

Val Phe Phe Leu Phe Ala Gly Val Leu Leu Ala Phe Thr Leu Phe Thr
 465 470 475 480

Phe Phe Lys Val Pro Glu Thr Lys Gly Lys Ser Phe Glu Glu Ile Ala
 485 490 495

Ala Glu Phe Gln Lys Lys Ser Gly Ser Ala His Arg Pro Lys Ala Ala
 500 505 510

Val Glu Met Lys Phe Leu Gly Ala Thr Glu Thr Val
 515 520

<210> 38
 <211> 3915
 <212> DNA
 <213> GLUT3

<220>
 <221> CDS
 <222> (243)..(1733)
 <223>

<400> 38
 gtgggggtggg gtgggggtggg gggcttgtcg ccctttcagg ctccaccctt tgcggagatt 60
 ataaatagtc atgatcccag cgagaccag agatgcctgt aatggtgaga ctttggatcc 120
 ttcttgagga cgtggagaaa actttctgct gagaaggaca ttttgaaggt tttgttggct 180
 gaaaaagctg tttctggaat caccctaga tctttcttga agacttgaat tagattacag 240
 cg atg ggg aca cag aag gtc acc cca gct ctg ata ttt gcc atc aca 287
 Met Gly Thr Gln Lys Val Thr Pro Ala Leu Ile Phe Ala Ile Thr
 1 5 10 15
 gtt gct aca atc ggc tct ttc caa ttt ggc tac aac act ggg gtc atc 335
 Val Ala Thr Ile Gly Ser Phe Gln Phe Gly Tyr Asn Thr Gly Val Ile
 20 25 30
 aat gct cct gag aag atc ata aag gaa ttt atc aat aaa act ttg acg 383
 Asn Ala Pro Glu Lys Ile Ile Lys Glu Phe Ile Asn Lys Thr Leu Thr
 35 40 45
 gac aag gga aat gcc cca ccc tct gag gtg ctg ctc acg tct ctc tgg 431
 Asp Lys Gly Asn Ala Pro Pro Ser Glu Val Leu Leu Thr Ser Leu Trp
 50 55 60

tcc ttg tct gtg gcc ata ttt tcc gtc ggg ggt atg atc ggc tcc ttt Ser Leu Ser Val Ala Ile Phe Ser Val Gly Gly Met Ile Gly Ser Phe 65 70 75	479
tcc gtc gga ctc ttc gtc aac cgc ttt ggc agg cgc aat tca atg ctg Ser Val Gly Leu Phe Val Asn Arg Phe Gly Arg Arg Asn Ser Met Leu 80 85 90 95	527
att gtc aac ctg ttg gct gtc act ggt ggc tgc ttt atg gga ctg tgt Ile Val Asn Leu Leu Ala Val Thr Gly Gly Cys Phe Met Gly Leu Cys 100 105 110	575
aaa gta gct aag tcg gtt gaa atg ctg atc ctg ggt cgc ttg gtt att Lys Val Ala Lys Ser Val Glu Met Leu Ile Leu Gly Arg Leu Val Ile 115 120 125	623
ggc ctc ttc tgc gga ctc tgc aca ggt ttt gtg ccc atg tac att gga Gly Leu Phe Cys Gly Leu Cys Thr Gly Phe Val Pro Met Tyr Ile Gly 130 135 140	671
gag atc tcg cct act gcc ctg cgg ggt gcc ttt ggc act ctc aac cag Glu Ile Ser Pro Thr Ala Leu Arg Gly Ala Phe Gly Thr Leu Asn Gln 145 150 155	719
ctg ggc atc gtt gtt gga att ctg gtg gcc cag atc ttt ggt ctg gaa Leu Gly Ile Val Val Gly Ile Leu Val Ala Gln Ile Phe Gly Leu Glu 160 165 170 175	767
ttc atc ctt ggg tct gaa gag cta tgg ccg ctg cta ctg ggt ttt acc Phe Ile Leu Gly Ser Glu Glu Leu Trp Pro Leu Leu Leu Gly Phe Thr 180 185 190	815
atc ctt cct gct atc cta caa agt gca gcc ctt cca ttt tgc cct gaa Ile Leu Pro Ala Ile Leu Gln Ser Ala Ala Leu Pro Phe Cys Pro Glu 195 200 205	863
agt ccc aga ttt ttg ctc att aac aga aaa gaa gag gag aat gct aag Ser Pro Arg Phe Leu Leu Ile Asn Arg Lys Glu Glu Glu Asn Ala Lys 210 215 220	911
cag atc ctc cag cgg ttg tgg ggc acc cag gat gta tcc caa gac atc Gln Ile Leu Gln Arg Leu Trp Gly Thr Gln Asp Val Ser Gln Asp Ile 225 230 235	959
cag gag atg aaa gat gag agt gca agg atg tca caa gaa aag caa gtc Gln Glu Met Lys Asp Glu Ser Ala Arg Met Ser Gln Glu Lys Gln Val 240 245 250 255	1007
acc gtg cta gag ctc ttt aga gtg tcc agc tac cga cag ccc atc atc Thr Val Leu Glu Leu Phe Arg Val Ser Ser Tyr Arg Gln Pro Ile Ile 260 265 270	1055
att tcc att gtg ctc cag ctc tct cag cag ctc tct ggg atc aat gct Ile Ser Ile Val Leu Gln Leu Ser Gln Gln Leu Ser Gly Ile Asn Ala 275 280 285	1103
gtg ttc tat tac tca aca gga atc ttc aag gat gca ggt gtt caa gag Val Phe Tyr Tyr Ser Thr Gly Ile Phe Lys Asp Ala Gly Val Gln Glu 290 295 300	1151
ccc atc tat gcc acc atc ggc gcg ggt gtg gtt aat act atc ttc act Pro Ile Tyr Ala Thr Ile Gly Ala Gly Val Val Asn Thr Ile Phe Thr	1199

305	310	315	
gta gtt tct cta ttt ctg gtg gaa agg gca gga aga agg act ctg cat			1247
Val Val Ser Leu Phe Leu Val Glu Arg Ala Gly Arg Arg Thr Leu His			
320	325	330	335
atg ata ggc ctt gga ggg atg gct ttt tgt tcc acg ctc atg act gtt			1295
Met Ile Gly Leu Gly Gly Met Ala Phe Cys Ser Thr Leu Met Thr Val			
	340	345	350
tct ttg tta tta aag gat aac tat aat ggg atg agc ttt gtc tgt att			1343
Ser Leu Leu Leu Lys Asp Asn Tyr Asn Gly Met Ser Phe Val Cys Ile			
	355	360	365
ggg gct atc ttg gtc ttt gta gcc ttc ttt gaa att gga cca ggc ccc			1391
Gly Ala Ile Leu Val Phe Val Ala Phe Phe Glu Ile Gly Pro Gly Pro			
	370	375	380
att ccc tgg ttt att gtg gcc gaa ctc ttc agc cag ggc ccc cgc cca			1439
Ile Pro Trp Phe Ile Val Ala Glu Leu Phe Ser Gln Gly Pro Arg Pro			
	385	390	395
gct gcg atg gca gtg gcc ggc tgc tcc aac tgg acc tcc aac ttc cta			1487
Ala Ala Met Ala Val Ala Gly Cys Ser Asn Trp Thr Ser Asn Phe Leu			
400	405	410	415
gtc gga ttg ctc ttc ccc tcc gct gct cac tat tta gga gcc tac gtt			1535
Val Gly Leu Leu Phe Pro Ser Ala Ala His Tyr Leu Gly Ala Tyr Val			
	420	425	430
ttt att atc ttc acc ggc ttc ctc att acc ttc ttg gct ttt acc ttc			1583
Phe Ile Ile Phe Thr Gly Phe Leu Ile Thr Phe Leu Ala Phe Thr Phe			
	435	440	445
ttc aaa gtc cct gag acc cgt ggc agg act ttt gag gat atc aca cgg			1631
Phe Lys Val Pro Glu Thr Arg Gly Arg Thr Phe Glu Asp Ile Thr Arg			
	450	455	460
gcc ttt gaa ggg cag gca cac ggt gca gat aga tct gga aag gac ggc			1679
Ala Phe Glu Gly Gln Ala His Gly Ala Asp Arg Ser Gly Lys Asp Gly			
465	470	475	
gtc atg gag atg aac agc atc gag cct gct aag gag acc acc acc aat			1727
Val Met Glu Met Asn Ser Ile Glu Pro Ala Lys Glu Thr Thr Thr Asn			
480	485	490	495
gtc taa gtcgtgcctc cttccacctc cctcccgga tgggaaagcc acctctccct			1783
Val			
caacaaggga gagacctcat caggatgaac ccaggacgct tctgaatgct gctacttaat			1843
tccttttctca tcccacgcac tccatgagca ccccaaggct gcggtttggt ggatcttcaa			1903
tggtttttta aattttatatt cctggacatc ctcttctgct taggagagac cgagtgaacc			1963
taccttcatt tcaggaggga ttggccgctt ggcacatgac aactttgcc gcttttccctc			2023
ccttggggttc tgatattgcc gcactagggg atataggaga ggaaaagtaa ggtgcagttc			2083
ccccaacctc agacttacca ggaagcagat acatatgagt gtggaagccg gaggtgtttt			2143
atgtaagagc accttcctca cttccataca gctctacgtg gcaaattaac ttgagtttta			2203

```

tttattttat cctctggttt aattacataa tttttttttt tttactttta gtttcaggat 2263
acatgtgccg aatgtgcagg tttgttacat aggtatataat atgccatgat ggaaatattt 2323
atTTTTTTaa gcgtaatttt gccaaataat aaaaacagaa ggaaattgag attagagggga 2383
ggtgttttaa gagaggttat agagtagaag atttgatgct ggagagggtta aggtgcaata 2443
agaatttagg gagaaatgtt gttcattatt ggagggtaaa tgatgtggtg cctgagggtct 2503
gtacgttacc tcttaacaat ttctgtcctt cagatggaaa ctctttaact tctcgtaaaa 2563
gtcatatacc tatataataa agctactgat ttccttggag cttttttctt taagataata 2623
gtttacatgt agtagtactt gaaatctagg attattaaact aatatgggca ttgtagttaa 2683
tgatggttga tgggttctaa ttttggtatg agtccaggga agagaaagtg atttctagaa 2743
agcctgttcc cctcactgga tgaaataact ccttcttgta gtagtctcat tacttttgaa 2803
gtaatccgc cactatctc gtgggagagc catccaaata agaaaccta aataattggt 2863
tcttggtaga gattcattat tttccactt tgttctttag gagattttag gtgttgattt 2923
tctgttgat ttaactcat acctttaaag gaattcccca aagaatgttt atagcaaact 2983
tggaatttgt aacctcagct ctgggagagg atttttttct gagcgattat tatctaaagt 3043
gtgttggtgc tttaggctca cggcacgctt gcgtatgtct gttaccatgt cactgtggtc 3103
ctatgccgaa tgccctcagg ggacttgaat ctttccaata aaccagggtt agacagtatg 3163
agtcaatgtg cagtgtagcc cacacttgag aggatgaatg tatgtgcaact gtcactttgc 3223
tctgggtgga agtacgttat tgttgactta ttttctctgt gtttgttctt acagcccctt 3283
tttcatatgt tgctcagtct ccctttccct tcttggtgct tacacatctc agacccttta 3343
gccaaaccct tgtcagtgc agtatTTTgg ttcttagttc tcactgttcc ctctgctcct 3403
ggagcctttg aataaaaatg cacgtagctg aggccggatg cgggtggctca cgcctgtaat 3463
cccagcactt tgggaggcct aggcgggcgg tcagggggtc gagaccagtc tggccaacat 3523
cgtgaaaccc tgtctctact aaaaatgcaa aaattagccg ggcgtggtgg cgggcgcctg 3583
taatcccagc tacttgggaa gctgaggcgg gagaatcatg tgaaccggg acgcagggtt 3643
tgcagtgagc ggagatcgca tcattgcact ctagcctggg ccacagggcg agactccgtc 3703
tcaaaaaaaa aaaaatgcac atagctatcg agtgtgctt agcttgaaaa ggtgaccttg 3763
caacttcatg tcaactttct ggctcctcaa acagtaggtt ggcagtaagg cagggtccca 3823
tttctcactg agaagattgt gaatatttcc atatggattt tctattgtta ctctggttct 3883
ttgttttaaa ataaaaattc tgaatgtaca cg 3915

```

<210> 39
<211> 496
<212> PRT

<213> GLUT3

<400> 39

```

Met Gly Thr Gln Lys Val Thr Pro Ala Leu Ile Phe Ala Ile Thr Val
1          5          10          15

Ala Thr Ile Gly Ser Phe Gln Phe Gly Tyr Asn Thr Gly Val Ile Asn
20          25          30

Ala Pro Glu Lys Ile Ile Lys Glu Phe Ile Asn Lys Thr Leu Thr Asp
35          40          45

Lys Gly Asn Ala Pro Pro Ser Glu Val Leu Leu Thr Ser Leu Trp Ser
50          55          60

Leu Ser Val Ala Ile Phe Ser Val Gly Gly Met Ile Gly Ser Phe Ser
65          70          75          80

Val Gly Leu Phe Val Asn Arg Phe Gly Arg Arg Asn Ser Met Leu Ile
85          90          95

Val Asn Leu Leu Ala Val Thr Gly Gly Cys Phe Met Gly Leu Cys Lys
100         105         110

Val Ala Lys Ser Val Glu Met Leu Ile Leu Gly Arg Leu Val Ile Gly
115         120         125

Leu Phe Cys Gly Leu Cys Thr Gly Phe Val Pro Met Tyr Ile Gly Glu
130         135         140

Ile Ser Pro Thr Ala Leu Arg Gly Ala Phe Gly Thr Leu Asn Gln Leu
145         150         155         160

Gly Ile Val Val Gly Ile Leu Val Ala Gln Ile Phe Gly Leu Glu Phe
165         170         175

Ile Leu Gly Ser Glu Glu Leu Trp Pro Leu Leu Leu Gly Phe Thr Ile
180         185         190

Leu Pro Ala Ile Leu Gln Ser Ala Ala Leu Pro Phe Cys Pro Glu Ser
195         200         205

Pro Arg Phe Leu Leu Ile Asn Arg Lys Glu Glu Glu Asn Ala Lys Gln
210         215         220

Ile Leu Gln Arg Leu Trp Gly Thr Gln Asp Val Ser Gln Asp Ile Gln
225         230         235         240

```

Glu Met Lys Asp Glu Ser Ala Arg Met Ser Gln Glu Lys Gln Val Thr
 245 250 255
 Val Leu Glu Leu Phe Arg Val Ser Ser Tyr Arg Gln Pro Ile Ile Ile
 260 265 270
 Ser Ile Val Leu Gln Leu Ser Gln Gln Leu Ser Gly Ile Asn Ala Val
 275 280 285
 Phe Tyr Tyr Ser Thr Gly Ile Phe Lys Asp Ala Gly Val Gln Glu Pro
 290 295 300
 Ile Tyr Ala Thr Ile Gly Ala Gly Val Val Asn Thr Ile Phe Thr Val
 305 310 315 320
 Val Ser Leu Phe Leu Val Glu Arg Ala Gly Arg Arg Thr Leu His Met
 325 330 335
 Ile Gly Leu Gly Gly Met Ala Phe Cys Ser Thr Leu Met Thr Val Ser
 340 345 350
 Leu Leu Leu Lys Asp Asn Tyr Asn Gly Met Ser Phe Val Cys Ile Gly
 355 360 365
 Ala Ile Leu Val Phe Val Ala Phe Phe Glu Ile Gly Pro Gly Pro Ile
 370 375 380
 Pro Trp Phe Ile Val Ala Glu Leu Phe Ser Gln Gly Pro Arg Pro Ala
 385 390 395 400
 Ala Met Ala Val Ala Gly Cys Ser Asn Trp Thr Ser Asn Phe Leu Val
 405 410 415
 Gly Leu Leu Phe Pro Ser Ala Ala His Tyr Leu Gly Ala Tyr Val Phe
 420 425 430
 Ile Ile Phe Thr Gly Phe Leu Ile Thr Phe Leu Ala Phe Thr Phe Phe
 435 440 445
 Lys Val Pro Glu Thr Arg Gly Arg Thr Phe Glu Asp Ile Thr Arg Ala
 450 455 460
 Phe Glu Gly Gln Ala His Gly Ala Asp Arg Ser Gly Lys Asp Gly Val
 465 470 475 480
 Met Glu Met Asn Ser Ile Glu Pro Ala Lys Glu Thr Thr Thr Asn Val
 485 490 495

<210> 40
 <211> 2218
 <212> DNA
 <213> GLUT5

<220>
 <221> CDS
 <222> (1) .. (1581)
 <223>

<400> 40
 ctt ctc tct cca ttc agt gca cgc gtt act ttg gct aaa agg agg tga 48
 Leu Leu Ser Pro Phe Ser Ala Arg Val Thr Leu Ala Lys Arg Arg
 1 5 10 15
 gcg gca ctc tgc cct tcc aga gca agc atg gag caa cag gat cag agc 96
 Ala Ala Leu Cys Pro Ser Arg Ala Ser Met Glu Gln Gln Asp Gln Ser
 20 25 30
 atg aag gaa ggg agg ctg acg ctt gtg ctt gcc ctg gca acc ctg ata 144
 Met Lys Glu Gly Arg Leu Thr Leu Val Leu Ala Leu Ala Thr Leu Ile
 35 40 45
 gct gcc ttt ggg tca tcc ttc cag tat ggg tac aac gtg gct gct gtc 192
 Ala Ala Phe Gly Ser Ser Phe Gln Tyr Gly Tyr Asn Val Ala Ala Val
 50 55 60
 aac tcc cca gca ctg ctc atg caa caa ttt tac aat gag act tac tat 240
 Asn Ser Pro Ala Leu Leu Met Gln Gln Phe Tyr Asn Glu Thr Tyr Tyr
 65 70 75
 ggt agg acc ggt gaa ttc atg gaa gac ttc ccc ttg acg ttg ctg tgg 288
 Gly Arg Thr Gly Glu Phe Met Glu Asp Phe Pro Leu Thr Leu Leu Trp
 80 85 90 95
 tct gta acc gtg tcc atg ttt cca ttt gga ggg ttt atc gga tcc ctc 336
 Ser Val Thr Val Ser Met Phe Pro Phe Gly Gly Phe Ile Gly Ser Leu
 100 105 110
 ctg gtc ggc ccc ttg gtg aat aaa ttt ggc aga aaa ggg gcc ttg ctg 384
 Leu Val Gly Pro Leu Val Asn Lys Phe Gly Arg Lys Gly Ala Leu Leu
 115 120 125
 ttc aac aac ata ttt tct atc gtg cct gcg atc tta atg gga tgc agc 432
 Phe Asn Asn Ile Phe Ser Ile Val Pro Ala Ile Leu Met Gly Cys Ser
 130 135 140
 aga gtc gcc aca tca ttt gag ctt atc att att tcc aga ctt ttg gtg 480
 Arg Val Ala Thr Ser Phe Glu Leu Ile Ile Ile Ser Arg Leu Leu Val
 145 150 155
 gga ata tgt gca ggt gta tct tcc aac gtg gtc ccc atg tac tta ggg 528
 Gly Ile Cys Ala Gly Val Ser Ser Asn Val Val Pro Met Tyr Leu Gly
 160 165 170 175
 gag ctg gcc cct aaa aac ctg cgg ggg gct ctc ggg gtg gtg ccc cag 576
 Glu Leu Ala Pro Lys Asn Leu Arg Gly Ala Leu Gly Val Val Pro Gln
 180 185 190
 ctc ttc atc act gtt ggc atc ctt gtg gcc cag atc ttt ggt ctt cgg 624
 Leu Phe Ile Thr Val Gly Ile Leu Val Ala Gln Ile Phe Gly Leu Arg

195	200	205	
aat ctc ctt gca aac gta gat ggc tgg ccg atc ctg ctg ggg ctg acc Asn Leu Leu Ala Asn Val Asp Gly Trp Pro Ile Leu Leu Gly Leu Thr 210 215 220			672
ggg gtc ccc gcg gcg ctg cag ctc ctt ctg ctg ccc ttc ttc ccc gag Gly Val Pro Ala Ala Leu Gln Leu Leu Leu Leu Pro Phe Phe Pro Glu 225 230 235			720
agc ccc agg tac ctg ctg att cag aag aaa gac gaa gcg gcc gcc aag Ser Pro Arg Tyr Leu Leu Ile Gln Lys Lys Asp Glu Ala Ala Ala Lys 240 245 250 255			768
aaa gcc cta cag acg ctg cgc ggc tgg gac tct gtg gac agg gag gtg Lys Ala Leu Gln Thr Leu Arg Gly Trp Asp Ser Val Asp Arg Glu Val 260 265 270			816
gcc gag atc cgg cag gag gat gag gca gag aag gcc gcg ggc ttc atc Ala Glu Ile Arg Gln Glu Asp Glu Ala Glu Lys Ala Ala Gly Phe Ile 275 280 285			864
tcc gtg ctg aag ctg ttc cgg atg cgc tcg ctg cgc tgg cag ctg ctg Ser Val Leu Lys Leu Phe Arg Met Arg Ser Leu Arg Trp Gln Leu Leu 290 295 300			912
tcc atc atc gtc ctc atg ggc ggc cag cag ctg tcg ggc gtc aac gct Ser Ile Ile Val Leu Met Gly Gln Gln Leu Ser Gly Val Asn Ala 305 310 315			960
atc tac tac tac gcg gac cag atc tac ctg agc gcc ggc gtg ccg gag Ile Tyr Tyr Tyr Ala Asp Gln Ile Tyr Leu Ser Ala Gly Val Pro Glu 320 325 330 335			1008
gag cac gtg cag tac gtg acg gcc ggc acc ggg gcc gtg aac gtg gtc Glu His Val Gln Tyr Val Thr Ala Gly Thr Gly Ala Val Asn Val Val 340 345 350			1056
atg acc ttc tgc gcc gtg ttc gtg gtg gag ctc ctg ggt cgg agg ctg Met Thr Phe Cys Ala Val Phe Val Val Glu Leu Leu Gly Arg Arg Leu 355 360 365			1104
ctg ctg ctg ctg ggc ttc tcc atc tgc ctc ata gcc tgc tgc gtg ctc Leu Leu Leu Leu Gly Phe Ser Ile Cys Leu Ile Ala Cys Cys Val Leu 370 375 380			1152
act gca gct ctg gca ctg cag gac aca gtg tcc tgg atg cca tac atc Thr Ala Ala Leu Ala Leu Gln Asp Thr Val Ser Trp Met Pro Tyr Ile 385 390 395			1200
agc atc gtc tgt gtc atc tcc tac gtc ata gga cat gcc ctc ggg ccc Ser Ile Val Cys Val Ile Ser Tyr Val Ile Gly His Ala Leu Gly Pro 400 405 410 415			1248
agt ccc ata ccc gcg ctg ctc atc act gag atc ttc ctg cag tcc tct Ser Pro Ile Pro Ala Leu Leu Ile Thr Glu Ile Phe Leu Gln Ser Ser 420 425 430			1296
cgg cca tct gcc ttc atg gtg ggg ggc agt gtg cac tgg ctc tcc aac Arg Pro Ser Ala Phe Met Val Gly Gly Ser Val His Trp Leu Ser Asn 435 440 445			1344
ttc acc gtg ggc ttg atc ttc ccg ttc atc cag gag ggc ctc ggc ccg			1392

Phe Thr Val Gly Leu Ile Phe Pro Phe Ile Gln Glu Gly Leu Gly Pro
 450 455 460
 tac agc ttc att gtc ttc gcc gtg atc tgc ctc ctc acc acc atc tac 1440
 Tyr Ser Phe Ile Val Phe Ala Val Ile Cys Leu Leu Thr Thr Ile Tyr
 465 470 475
 atc ttc ttg att gtc ccg gag acc aag gcc aag acg ttc ata gag atc 1488
 Ile Phe Leu Ile Val Pro Glu Thr Lys Ala Lys Thr Phe Ile Glu Ile
 480 485 490 495
 aac cag att ttc acc aag atg aat aag gtg tct gaa gtg tac ccg gaa 1536
 Asn Gln Ile Phe Thr Lys Met Asn Lys Val Ser Glu Val Tyr Pro Glu
 500 505 510
 aag gag gaa ctg aaa gag ctt cca cct gtc act tcg gaa cag tga 1581
 Lys Glu Glu Leu Lys Glu Leu Pro Pro Val Thr Ser Glu Gln
 515 520 525
 ctctggagag gaagccagtg gagctggtct gccaggggct tcccactttg gcttattttt 1641
 ctgacttcta gctgtctgtg aatatccaga aataaaacaa ctctgatgtg gaatgcagtc 1701
 ctcatctcca gcctccccac ccagtgaggga actgtgcaaa gggctgcctt gctgttcttg 1761
 aagctgggct gtctctctcc atgttggcct gtcaccagac ccgagtcaat taaacagctg 1821
 gtcctccact ttgtctgttc agccttcgtg tggctcctgg taacgtggct ccaccttgat 1881
 ggggtcaacct ttgtgtggct cctggttaaca taacaacaac agttactata gtggtgagat 1941
 ggaaggaatc aaattttgcc agagaaacta actcgggtggc cccaacaggt cttccggggc 2001
 catgggcatt tgttttagagc caaattcatc ctcttaccag atccttttcc agaaatacct 2061
 gtctaggaag gtgtgatgtc agaaacaatg acatccagaa agctgaggaa caggttcctg 2121
 tggagacact gagtcagaat tcttcatcca aattattttg ttagtggaat atggaattgc 2181
 ttctgtgtag tcaataaaat gaacctgac acttttc 2218

<210> 41
 <211> 15
 <212> PRT
 <213> GLUT5

<400> 41

Leu Leu Ser Pro Phe Ser Ala Arg Val Thr Leu Ala Lys Arg Arg
 1 5 10 15

<210> 42
 <211> 510
 <212> PRT
 <213> GLUT5

<400> 42

Ala Ala Leu Cys Pro Ser Arg Ala Ser Met Glu Gln Gln Asp Gln Ser
 1 5 10 15

Met Lys Glu Gly Arg Leu Thr Leu Val Leu Ala Leu Ala Thr Leu Ile
 20 25 30

Ala Ala Phe Gly Ser Ser Phe Gln Tyr Gly Tyr Asn Val Ala Ala Val
 35 40 45

Asn Ser Pro Ala Leu Leu Met Gln Gln Phe Tyr Asn Glu Thr Tyr Tyr
 50 55 60

Gly Arg Thr Gly Glu Phe Met Glu Asp Phe Pro Leu Thr Leu Leu Trp
 65 70 75 80

Ser Val Thr Val Ser Met Phe Pro Phe Gly Gly Phe Ile Gly Ser Leu
 85 90 95

Leu Val Gly Pro Leu Val Asn Lys Phe Gly Arg Lys Gly Ala Leu Leu
 100 105 110

Phe Asn Asn Ile Phe Ser Ile Val Pro Ala Ile Leu Met Gly Cys Ser
 115 120 125

Arg Val Ala Thr Ser Phe Glu Leu Ile Ile Ile Ser Arg Leu Leu Val
 130 135 140

Gly Ile Cys Ala Gly Val Ser Ser Asn Val Val Pro Met Tyr Leu Gly
 145 150 155 160

Glu Leu Ala Pro Lys Asn Leu Arg Gly Ala Leu Gly Val Val Pro Gln
 165 170 175

Leu Phe Ile Thr Val Gly Ile Leu Val Ala Gln Ile Phe Gly Leu Arg
 180 185 190

Asn Leu Leu Ala Asn Val Asp Gly Trp Pro Ile Leu Leu Gly Leu Thr
 195 200 205

Gly Val Pro Ala Ala Leu Gln Leu Leu Leu Leu Pro Phe Phe Pro Glu
 210 215 220

Ser Pro Arg Tyr Leu Leu Ile Gln Lys Lys Asp Glu Ala Ala Ala Lys
 225 230 235 240

Lys Ala Leu Gln Thr Leu Arg Gly Trp Asp Ser Val Asp Arg Glu Val
 245 250 255

Ala Glu Ile Arg Gln Glu Asp Glu Ala Glu Lys Ala Ala Gly Phe Ile
 260 265 270

80

Ser Val Leu Lys Leu Phe Arg Met Arg Ser Leu Arg Trp Gln Leu Leu
 275 280 285

Ser Ile Ile Val Leu Met Gly Gly Gln Gln Leu Ser Gly Val Asn Ala
 290 295 300

Ile Tyr Tyr Tyr Ala Asp Gln Ile Tyr Leu Ser Ala Gly Val Pro Glu
 305 310 315 320

Glu His Val Gln Tyr Val Thr Ala Gly Thr Gly Ala Val Asn Val Val
 325 330 335

Met Thr Phe Cys Ala Val Phe Val Val Glu Leu Leu Gly Arg Arg Leu
 340 345 350

Leu Leu Leu Leu Gly Phe Ser Ile Cys Leu Ile Ala Cys Cys Val Leu
 355 360 365

Thr Ala Ala Leu Ala Leu Gln Asp Thr Val Ser Trp Met Pro Tyr Ile
 370 375 380

Ser Ile Val Cys Val Ile Ser Tyr Val Ile Gly His Ala Leu Gly Pro
 385 390 395 400

Ser Pro Ile Pro Ala Leu Leu Ile Thr Glu Ile Phe Leu Gln Ser Ser
 405 410 415

Arg Pro Ser Ala Phe Met Val Gly Gly Ser Val His Trp Leu Ser Asn
 420 425 430

Phe Thr Val Gly Leu Ile Phe Pro Phe Ile Gln Glu Gly Leu Gly Pro
 435 440 445

Tyr Ser Phe Ile Val Phe Ala Val Ile Cys Leu Leu Thr Thr Ile Tyr
 450 455 460

Ile Phe Leu Ile Val Pro Glu Thr Lys Ala Lys Thr Phe Ile Glu Ile
 465 470 475 480

Asn Gln Ile Phe Thr Lys Met Asn Lys Val Ser Glu Val Tyr Pro Glu
 485 490 495

Lys Glu Glu Leu Lys Glu Leu Pro Pro Val Thr Ser Glu Gln
 500 505 510

<210> 43

<211> 1580
 <212> DNA
 <213> GLUT6

<220>
 <221> CDS
 <222> (47)..(1570)
 <223>

<400> 43

```

tgctccagtc tgagcgccct ccgctcgccc cgagagagac ccggcc atg cag gag      55
                                     Met Gln Glu
                                     1

ccg ctg ctg gga gcc gag ggc ccg gac tac gac acc ttc ccc gag aag      103
Pro Leu Leu Gly Ala Glu Gly Pro Asp Tyr Asp Thr Phe Pro Glu Lys
  5                               10                               15

ccg ccc ccg tcg cca ggg gac agg gcg cgg gtc ggg acc ctg cag aac      151
Pro Pro Pro Ser Pro Gly Asp Arg Ala Arg Val Gly Thr Leu Gln Asn
 20                               25                               30                               35

aaa agg gtg ttc ctg gcc acc ttc gcc gca gtg ctc ggc aat ttc agc      199
Lys Arg Val Phe Leu Ala Thr Phe Ala Ala Val Leu Gly Asn Phe Ser
                               40                               45                               50

ttt ggg tat gcc ctg gtc tac aca tcc cct gtc atc cca gcc ctg gag      247
Phe Gly Tyr Ala Leu Val Tyr Thr Ser Pro Val Ile Pro Ala Leu Glu
                               55                               60                               65

cgc tcc ttg gat cct gac ctg cat ctg acc aaa tcc cag gca tcc tgg      295
Arg Ser Leu Asp Pro Asp Leu His Leu Thr Lys Ser Gln Ala Ser Trp
 70                               75                               80

ttt ggg tcc gtg ttc acc ctg gga gca gcg gcc gga ggc ctg agt gcc      343
Phe Gly Ser Val Phe Thr Leu Gly Ala Ala Ala Gly Gly Leu Ser Ala
 85                               90                               95

atg atc ctc aac gac ctc ctg ggc cgg aag ctg agc atc atg ttc tca      391
Met Ile Leu Asn Asp Leu Leu Gly Arg Lys Leu Ser Ile Met Phe Ser
100                               105                               110                               115

gct gtg ccg tcg gcg gcc ggc tat gcg ctc atg gcg ggt gcg cac ggc      439
Ala Val Pro Ser Ala Ala Gly Tyr Ala Leu Met Ala Gly Ala His Gly
120                               125                               130

ctc tgg atg ctg ctg ctc gga agg acg ctg acg ggc ttc gcc ggg ggg      487
Leu Trp Met Leu Leu Leu Gly Arg Thr Leu Thr Gly Phe Ala Gly Gly
135                               140                               145

ctc aca gct gcc tgc atc ccg gtg tac gtg tct gag att gct ccc cca      535
Leu Thr Ala Ala Cys Ile Pro Val Tyr Val Ser Glu Ile Ala Pro Pro
150                               155                               160

ggc gtt cgt ggg gct ctg ggg gcc aca ccc cag ctc atg gca gtg ttc      583
Gly Val Arg Gly Ala Leu Gly Ala Thr Pro Gln Leu Met Ala Val Phe
165                               170                               175

gga tcc ctg tcc ctc tac gcc ctt ggc ctc ctg ctg ccg tgg cgc tgg      631
Gly Ser Leu Ser Leu Tyr Ala Leu Gly Leu Leu Leu Pro Trp Arg Trp
180                               185                               190                               195

ctg gct gtg gcc ggg gag gcg cct gtg ctc atc atg atc ctg ctg ctc      679

```

Leu	Ala	Val	Ala	Gly	Glu	Ala	Pro	Val	Leu	Ile	Met	Ile	Leu	Leu	Leu		
				200					205					210			
agc	ttc	atg	ccc	aac	tcg	ccg	cgc	ttc	ctg	ctc	tct	cgg	ggc	agg	gac	727	
Ser	Phe	Met	Pro	Asn	Ser	Pro	Arg	Phe	Leu	Leu	Ser	Arg	Gly	Arg	Asp		
			215					220					225				
gaa	gag	gcc	ctg	cgg	gcg	ctg	gcc	tgg	ctg	cgt	ggg	acg	gac	gtc	gat	775	
Glu	Glu	Ala	Leu	Arg	Ala	Leu	Ala	Trp	Leu	Arg	Gly	Thr	Asp	Val	Asp		
		230					235					240					
gtc	cac	tgg	gag	ttc	gag	cag	atc	cag	gac	aac	gtc	cgg	aga	cag	agc	823	
Val	His	Trp	Glu	Phe	Glu	Gln	Ile	Gln	Asp	Asn	Val	Arg	Arg	Gln	Ser		
	245					250					255						
agc	cga	gta	tcg	tgg	gct	gag	gca	cgg	gcc	cca	cac	gtg	tgc	cgg	ccc	871	
Ser	Arg	Val	Ser	Trp	Ala	Glu	Ala	Arg	Ala	Pro	His	Val	Cys	Arg	Pro		
260					265					270					275		
atc	acc	gtg	gcc	ttg	ctg	atg	cgc	ctc	ctg	cag	cag	ctg	acg	ggc	atc	919	
Ile	Thr	Val	Ala	Leu	Leu	Met	Arg	Leu	Leu	Gln	Gln	Leu	Thr	Gly	Ile		
				280					285					290			
acg	ccc	atc	ctg	gtc	tac	ctg	cag	tcc	atc	ttc	gac	agc	acc	gct	gtc	967	
Thr	Pro	Ile	Leu	Val	Tyr	Leu	Gln	Ser	Ile	Phe	Asp	Ser	Thr	Ala	Val		
			295					300					305				
ctg	ctg	ccc	ccc	aag	gac	gac	gca	gcc	atc	gtt	ggg	gcc	gtg	cgg	ctc	1015	
Leu	Leu	Pro	Pro	Lys	Asp	Asp	Ala	Ala	Ile	Val	Gly	Ala	Val	Arg	Leu		
		310					315					320					
ctg	tcc	gtg	ctg	atc	gcc	gcc	ctc	acc	atg	gac	ctc	gca	ggc	cgc	aag	1063	
Leu	Ser	Val	Leu	Ile	Ala	Ala	Leu	Thr	Met	Asp	Leu	Ala	Gly	Arg	Lys		
	325					330					335						
gtg	ctg	ctc	ttc	gtc	tca	gcg	gcc	atc	atg	ttt	gct	gcc	aac	ctg	act	1111	
Val	Leu	Leu	Phe	Val	Ser	Ala	Ala	Ile	Met	Phe	Ala	Ala	Asn	Leu	Thr		
340					345				350					355			
ctg	ggg	ctg	tac	atc	cac	ttt	ggc	ccc	agg	cct	ctg	agc	ccc	aac	agc	1159	
Leu	Gly	Leu	Tyr	Ile	His	Phe	Gly	Pro	Arg	Pro	Leu	Ser	Pro	Asn	Ser		
				360				365						370			
act	gcg	ggc	ctg	gaa	agc	gag	tcc	tgg	ggg	gac	ttg	gcg	cag	ccc	ctg	1207	
Thr	Ala	Gly	Leu	Glu	Ser	Glu	Ser	Trp	Gly	Asp	Leu	Ala	Gln	Pro	Leu		
			375					380					385				
gca	gca	ccc	gct	ggc	tac	ctc	acc	ctg	gtg	ccc	ctg	ctg	gcc	acc	atg	1255	
Ala	Ala	Pro	Ala	Gly	Tyr	Leu	Thr	Leu	Val	Pro	Leu	Leu	Ala	Thr	Met		
		390					395					400					
ctc	ttc	atc	atg	ggc	tac	gcc	gtg	ggc	tgg	ggt	ccc	atc	acc	tgg	ctg	1303	
Leu	Phe	Ile	Met	Gly	Tyr	Ala	Val	Gly	Trp	Gly	Pro	Ile	Thr	Trp	Leu		
	405					410					415						
ctc	atg	tct	gag	gtc	ctg	ccc	ctg	cgt	gcc	cgt	ggc	gtg	gcc	tca	ggg	1351	
Leu	Met	Ser	Glu	Val	Leu	Pro	Leu	Arg	Ala	Arg	Gly	Val	Ala	Ser	Gly		
420					425				430					435			
ctc	tgc	gtg	ctg	gcc	agc	tgg	ctc	acc	gcc	ttc	gtc	ctc	acc	aag	tcc	1399	
Leu	Cys	Val	Leu	Ala	Ser	Trp	Leu	Thr	Ala	Phe	Val	Leu	Thr	Lys	Ser		
				440					445					450			

ttc ctg cca gtg gtg agc acc ttc ggc ctc cag gtg cct ttc ttc ttc 1447
 Phe Leu Pro Val Val Ser Thr Phe Gly Leu Gln Val Pro Phe Phe Phe
 455 460 465

ttc gcg gcc atc tgc ttg gtg agc ctg gtg ttc aca ggc tgc tgt gtg 1495
 Phe Ala Ala Ile Cys Leu Val Ser Leu Val Phe Thr Gly Cys Cys Val
 470 475 480

ccc gag acc aag gga cgg tcc ctg gag cag atc gag tcc ttc ttc cgc 1543
 Pro Glu Thr Lys Gly Arg Ser Leu Glu Gln Ile Glu Ser Phe Phe Arg
 485 490 495

atg ggg aga agg tcc ttc ttg cgc tag gtcaagggtcc 1580
 Met Gly Arg Arg Ser Phe Leu Arg
 500 505

<210> 44
 <211> 507
 <212> PRT
 <213> GLUT6

<400> 44

Met Gln Glu Pro Leu Leu Gly Ala Glu Gly Pro Asp Tyr Asp Thr Phe
 1 5 10 15

Pro Glu Lys Pro Pro Pro Ser Pro Gly Asp Arg Ala Arg Val Gly Thr
 20 25 30

Leu Gln Asn Lys Arg Val Phe Leu Ala Thr Phe Ala Ala Val Leu Gly
 35 40 45

Asn Phe Ser Phe Gly Tyr Ala Leu Val Tyr Thr Ser Pro Val Ile Pro
 50 55 60

Ala Leu Glu Arg Ser Leu Asp Pro Asp Leu His Leu Thr Lys Ser Gln
 65 70 75 80

Ala Ser Trp Phe Gly Ser Val Phe Thr Leu Gly Ala Ala Ala Gly Gly
 85 90 95

Leu Ser Ala Met Ile Leu Asn Asp Leu Leu Gly Arg Lys Leu Ser Ile
 100 105 110

Met Phe Ser Ala Val Pro Ser Ala Ala Gly Tyr Ala Leu Met Ala Gly
 115 120 125

Ala His Gly Leu Trp Met Leu Leu Leu Gly Arg Thr Leu Thr Gly Phe
 130 135 140

Ala Gly Gly Leu Thr Ala Ala Cys Ile Pro Val Tyr Val Ser Glu Ile
 145 150 155 160

Ala Pro Pro Gly Val Arg Gly Ala Leu Gly Ala Thr Pro Gln Leu Met
 165 170 175

Ala Val Phe Gly Ser Leu Ser Leu Tyr Ala Leu Gly Leu Leu Leu Pro
 180 185 190

Trp Arg Trp Leu Ala Val Ala Gly Glu Ala Pro Val Leu Ile Met Ile
 195 200 205

Leu Leu Leu Ser Phe Met Pro Asn Ser Pro Arg Phe Leu Leu Ser Arg
 210 215 220

Gly Arg Asp Glu Glu Ala Leu Arg Ala Leu Ala Trp Leu Arg Gly Thr
 225 230 235 240

Asp Val Asp Val His Trp Glu Phe Glu Gln Ile Gln Asp Asn Val Arg
 245 250 255

Arg Gln Ser Ser Arg Val Ser Trp Ala Glu Ala Arg Ala Pro His Val
 260 265 270

Cys Arg Pro Ile Thr Val Ala Leu Leu Met Arg Leu Leu Gln Gln Leu
 275 280 285

Thr Gly Ile Thr Pro Ile Leu Val Tyr Leu Gln Ser Ile Phe Asp Ser
 290 295 300

Thr Ala Val Leu Leu Pro Pro Lys Asp Asp Ala Ala Ile Val Gly Ala
 305 310 315 320

Val Arg Leu Leu Ser Val Leu Ile Ala Ala Leu Thr Met Asp Leu Ala
 325 330 335

Gly Arg Lys Val Leu Leu Phe Val Ser Ala Ala Ile Met Phe Ala Ala
 340 345 350

Asn Leu Thr Leu Gly Leu Tyr Ile His Phe Gly Pro Arg Pro Leu Ser
 355 360 365

Pro Asn Ser Thr Ala Gly Leu Glu Ser Glu Ser Trp Gly Asp Leu Ala
 370 375 380

Gln Pro Leu Ala Ala Pro Ala Gly Tyr Leu Thr Leu Val Pro Leu Leu
 385 390 395 400

Ala Thr Met Leu Phe Ile Met Gly Tyr Ala Val Gly Trp Gly Pro Ile
 405 410 415

Thr Trp Leu Leu Met Ser Glu Val Leu Pro Leu Arg Ala Arg Gly Val
420 425 430

Ala Ser Gly Leu Cys Val Leu Ala Ser Trp Leu Thr Ala Phe Val Leu
435 440 445

Thr Lys Ser Phe Leu Pro Val Val Ser Thr Phe Gly Leu Gln Val Pro
450 455 460

Phe Phe Phe Phe Ala Ala Ile Cys Leu Val Ser Leu Val Phe Thr Gly
465 470 475 480

Cys Cys Val Pro Glu Thr Lys Gly Arg Ser Leu Glu Gln Ile Glu Ser
485 490 495

Phe Phe Arg Met Gly Arg Arg Ser Phe Leu Arg
500 505

<210> 45
<211> 1575
<212> DNA
<213> GLUT7

<220> .
<221> CDS
<222> (1)..(1575)
<223>

<400> 45
atg gag aac aaa gag gcg gga acc cct cca ccc att cca tcc agg gag 48
Met Glu Asn Lys Glu Ala Gly Thr Pro Pro Pro Ile Pro Ser Arg Glu
1 5 10 15
ggg cgg ctc cag ccg acg ctg ttg ctg gcg aca ctg agc gcg gcc ttt 96
Gly Arg Leu Gln Pro Thr Leu Leu Leu Ala Thr Leu Ser Ala Ala Phe
20 25 30
ggc tca gcc ttc cag tac ggc tac aac ctc tct gtg gtc aac acg ccg 144
Gly Ser Ala Phe Gln Tyr Gly Tyr Asn Leu Ser Val Val Asn Thr Pro
35 40 45
cac aag gtg ggc aca agc tgt gga tgg ggc aat gtt ttc cag gtc ttc 192
His Lys Val Gly Thr Ser Cys Gly Trp Gly Asn Val Phe Gln Val Phe
50 55 60
aag tca ttt tac aac gaa acc tac ttt gag cga cac gca aca ttc atg 240
Lys Ser Phe Tyr Asn Glu Thr Tyr Phe Glu Arg His Ala Thr Phe Met
65 70 75 80
gac ggg aag ctc atg ctg ctt cta tgg tct tgc acc gtc tcc atg ttt 288
Asp Gly Lys Leu Met Leu Leu Leu Trp Ser Cys Thr Val Ser Met Phe
85 90 95
cct ctg ggc ggc ctg ttg ggg tca ttg ctc gtg ggc ctg ctg gtt gat 336
Pro Leu Gly Gly Leu Leu Gly Ser Leu Leu Val Gly Leu Leu Val Asp

100	105	110	
agc tgc ggc aga aag ggg acc ctg ctg atc aac aac atc ttt gcc atc Ser Cys Gly Arg Lys Gly Thr Leu Leu Ile Asn Asn Ile Phe Ala Ile 115 120 125			384
atc ccc gcc atc ctg atg gga gtc agc aaa gtg gcc aag gct ttt gag Ile Pro Ala Ile Leu Met Gly Val Ser Lys Val Ala Lys Ala Phe Glu 130 135 140			432
ctg atc gtc ttt tcc cga gtg gtg ctg gga gtc tgt gca ggc atc tcc Leu Ile Val Phe Ser Arg Val Val Leu Gly Val Cys Ala Gly Ile Ser 145 150 155 160			480
tac agc gcc ctt ccc atg tac ctg gga gaa ctg gcc ccc aag aac ctg Tyr Ser Ala Leu Pro Met Tyr Leu Gly Glu Leu Ala Pro Lys Asn Leu 165 170 175			528
aga ggc atg gtg gga aca atg acc gag gtt ttc gtc atc gtt gga gtc Arg Gly Met Val Gly Thr Met Thr Glu Val Phe Val Ile Val Gly Val 180 185 190			576
ttc cta gca cag atc ttc agc ctc cag gcc atc ttg ggc aac ccg gca Phe Leu Ala Gln Ile Phe Ser Leu Gln Ala Ile Leu Gly Asn Pro Ala 195 200 205			624
ggc tgg ccg gtg ctt ctg gcg ctc aca ggg gtg ccc gcc ctg ctg cag Gly Trp Pro Val Leu Leu Ala Leu Thr Gly Val Pro Ala Leu Leu Gln 210 215 220			672
ctg ctg acc ctg ccc ttc ttc ccc gaa agc ccc cgc tac tcc ctg att Leu Leu Thr Leu Pro Phe Phe Pro Glu Ser Pro Arg Tyr Ser Leu Ile 225 230 235 240			720
cag aaa gga gat gaa gcc aca gcg cga caa gct ctg agg agg ctg aga Gln Lys Gly Asp Glu Ala Thr Ala Arg Gln Ala Leu Arg Arg Leu Arg 245 250 255			768
ggc cac acg gac atg gag gcc gag ctg gag gac atg cgt gcg gag gcc Gly His Thr Asp Met Glu Ala Glu Leu Glu Asp Met Arg Ala Glu Ala 260 265 270			816
cgg gcc gag cgc gcc gag ggc cac ctg tct gtg ctg cac ctc tgt gcc Arg Ala Glu Arg Ala Glu Gly His Leu Ser Val Leu His Leu Cys Ala 275 280 285			864
ctg cgg tcc ctg cgc tgg cag ctc ctc tcc atc atc gtg ctc atg gcc Leu Arg Ser Leu Arg Trp Gln Leu Leu Ser Ile Ile Val Leu Met Ala 290 295 300			912
ggc cag cag ctg tcg ggc atc aat gcg atc aac tac tat gcg gac acc Gly Gln Gln Leu Ser Gly Ile Asn Ala Ile Asn Tyr Tyr Ala Asp Thr 305 310 315 320			960
atc tac aca tct gcg ggc gtg gag gcc gct cac tcc caa tat gta acg Ile Tyr Thr Ser Ala Gly Val Glu Ala Ala His Ser Gln Tyr Val Thr 325 330 335			1008
gtg ggc tct ggc gtc gtc aac ata gtg atg acc atc acc tcg gct gtc Val Gly Ser Gly Val Val Asn Ile Val Met Thr Ile Thr Ser Ala Val 340 345 350			1056
ctt gtg gag cgg ctg gga cgg cgg cac ctc ctg ctg gcc ggc tac ggc			1104

Leu Val Glu Arg Leu Gly Arg Arg His Leu Leu Leu Ala Gly Tyr Gly
 355 360 365
 atc tgc ggc tct gcc tgc ctg gtg ctg acg gtg gtg ctc cta ttc cag 1152
 Ile Cys Gly Ser Ala Cys Leu Val Leu Thr Val Val Leu Leu Phe Gln
 370 375 380
 aac agg gtc ccc gag ctg tcc tac ctc ggc atc atc tgt gtc ttt gcc 1200
 Asn Arg Val Pro Glu Leu Ser Tyr Leu Gly Ile Ile Cys Val Phe Ala
 385 390 395 400
 tac atc gcg gga cat tcc att ggg ccc agt cct gtc ccc tcg gtg gtg 1248
 Tyr Ile Ala Gly His Ser Ile Gly Pro Ser Pro Val Pro Ser Val Val
 405 410 415
 agg acc gag atc ttc ctg cag tcc tcc cgg cgg gca gct ttc atg gtg 1296
 Arg Thr Glu Ile Phe Leu Gln Ser Ser Arg Arg Ala Ala Phe Met Val
 420 425 430
 gac ggg gca gtg cac tgg ctc acc aac ttc atc ata ggc ttc ctg ttc 1344
 Asp Gly Ala Val His Trp Leu Thr Asn Phe Ile Ile Gly Phe Leu Phe
 435 440 445
 cca tcc atc cag gag gcc atc ggt gcc tac agt ttc atc atc ttt gcc 1392
 Pro Ser Ile Gln Glu Ala Ile Gly Ala Tyr Ser Phe Ile Ile Phe Ala
 450 455 460
 gga atc tgc ctc ctc act gcg att tac atc tac gtg gtt att ccg gag 1440
 Gly Ile Cys Leu Leu Thr Ala Ile Tyr Ile Tyr Val Val Ile Pro Glu
 465 470 475 480
 acc aag ggc aaa aca ttt gtg gag ata aac cgc att ttt gcc aag aga 1488
 Thr Lys Gly Lys Thr Phe Val Glu Ile Asn Arg Ile Phe Ala Lys Arg
 485 490 495
 aac agg gtg aag ctt cca gag gag aaa gaa gaa acc att gat gct ggg 1536
 Asn Arg Val Lys Leu Pro Glu Glu Lys Glu Glu Thr Ile Asp Ala Gly
 500 505 510
 cct ccc aca gcc tct cct gcc aag gaa act tcc ttt tag 1575
 Pro Pro Thr Ala Ser Pro Ala Glu Thr Ser Phe
 515 520
 <210> 46
 <211> 524
 <212> PRT
 <213> GLUT7
 <400> 46
 Met Glu Asn Lys Glu Ala Gly Thr Pro Pro Pro Ile Pro Ser Arg Glu
 1 5 10 15
 Gly Arg Leu Gln Pro Thr Leu Leu Leu Ala Thr Leu Ser Ala Ala Phe
 20 25 30
 Gly Ser Ala Phe Gln Tyr Gly Tyr Asn Leu Ser Val Val Asn Thr Pro
 35 40 45

His Lys Val Gly Thr Ser Cys Gly Trp Gly Asn Val Phe Gln Val Phe
 50 55 60

Lys Ser Phe Tyr Asn Glu Thr Tyr Phe Glu Arg His Ala Thr Phe Met
 65 70 75 80

Asp Gly Lys Leu Met Leu Leu Leu Trp Ser Cys Thr Val Ser Met Phe
 85 90 95

Pro Leu Gly Gly Leu Leu Gly Ser Leu Leu Val Gly Leu Leu Val Asp
 100 105 110

Ser Cys Gly Arg Lys Gly Thr Leu Leu Ile Asn Asn Ile Phe Ala Ile
 115 120 125

Ile Pro Ala Ile Leu Met Gly Val Ser Lys Val Ala Lys Ala Phe Glu
 130 135 140

Leu Ile Val Phe Ser Arg Val Val Leu Gly Val Cys Ala Gly Ile Ser
 145 150 155 160

Tyr Ser Ala Leu Pro Met Tyr Leu Gly Glu Leu Ala Pro Lys Asn Leu
 165 170 175

Arg Gly Met Val Gly Thr Met Thr Glu Val Phe Val Ile Val Gly Val
 180 185 190

Phe Leu Ala Gln Ile Phe Ser Leu Gln Ala Ile Leu Gly Asn Pro Ala
 195 200 205

Gly Trp Pro Val Leu Leu Ala Leu Thr Gly Val Pro Ala Leu Leu Gln
 210 215 220

Leu Leu Thr Leu Pro Phe Phe Pro Glu Ser Pro Arg Tyr Ser Leu Ile
 225 230 235 240

Gln Lys Gly Asp Glu Ala Thr Ala Arg Gln Ala Leu Arg Arg Leu Arg
 245 250 255

Gly His Thr Asp Met Glu Ala Glu Leu Glu Asp Met Arg Ala Glu Ala
 260 265 270

Arg Ala Glu Arg Ala Glu Gly His Leu Ser Val Leu His Leu Cys Ala
 275 280 285

Leu Arg Ser Leu Arg Trp Gln Leu Leu Ser Ile Ile Val Leu Met Ala
 290 295 300

89

Gly Gln Gln Leu Ser Gly Ile Asn Ala Ile Asn Tyr Tyr Ala Asp Thr
 305 310 315 320

Ile Tyr Thr Ser Ala Gly Val Glu Ala Ala His Ser Gln Tyr Val Thr
 325 330 335

Val Gly Ser Gly Val Val Asn Ile Val Met Thr Ile Thr Ser Ala Val
 340 345 350

Leu Val Glu Arg Leu Gly Arg Arg His Leu Leu Leu Ala Gly Tyr Gly
 355 360 365

Ile Cys Gly Ser Ala Cys Leu Val Leu Thr Val Val Leu Leu Phe Gln
 370 375 380

Asn Arg Val Pro Glu Leu Ser Tyr Leu Gly Ile Ile Cys Val Phe Ala
 385 390 395 400

Tyr Ile Ala Gly His Ser Ile Gly Pro Ser Pro Val Pro Ser Val Val
 405 410 415

Arg Thr Glu Ile Phe Leu Gln Ser Ser Arg Arg Ala Ala Phe Met Val
 420 425 430

Asp Gly Ala Val His Trp Leu Thr Asn Phe Ile Ile Gly Phe Leu Phe
 435 440 445

Pro Ser Ile Gln Glu Ala Ile Gly Ala Tyr Ser Phe Ile Ile Phe Ala
 450 455 460

Gly Ile Cys Leu Leu Thr Ala Ile Tyr Ile Tyr Val Val Ile Pro Glu
 465 470 475 480

Thr Lys Gly Lys Thr Phe Val Glu Ile Asn Arg Ile Phe Ala Lys Arg
 485 490 495

Asn Arg Val Lys Leu Pro Glu Glu Lys Glu Glu Thr Ile Asp Ala Gly
 500 505 510

Pro Pro Thr Ala Ser Pro Ala Lys Glu Thr Ser Phe
 515 520

<210> 47
 <211> 1508
 <212> DNA
 <213> GLUT8

<220>

<221> CDS

<222> (27)..(1460)

<223>

<400> 47

```

agctggccga tgcgttgacc gccgac atg acg ccc gag gac cca gag gaa acc      53
                        Met Thr Pro Glu Asp Pro Glu Glu Thr
                        1                      5

cag ccg ctt ctg ggg cct cct ggc ggc agc gcg ccc cgc ggc cgc cgc      101
Gln Pro Leu Leu Gly Pro Pro Gly Gly Ser Ala Pro Arg Gly Arg Arg
10                      15                      20                      25

gtc ttc ctc gcc gcc ttc gcc gct gcc ctg ggc cca ctc agc ttc ggc      149
Val Phe Leu Ala Ala Phe Ala Ala Leu Gly Pro Leu Ser Phe Gly
                      30                      35                      40

ttc gcg ctc ggc tac agc tcc ccg gcc atc cct agc ctg cag cgc gcc      197
Phe Ala Leu Gly Tyr Ser Ser Pro Ala Ile Pro Ser Leu Gln Arg Ala
                      45                      50                      55

gcg ccc ccg gcc ccg cgc ctg gac gac gcc gcc gcc tcc tgg ttc ggg      245
Ala Pro Pro Ala Pro Arg Leu Asp Asp Ala Ala Ala Ser Trp Phe Gly
                      60                      65                      70

gct gtc gtg acc ctg ggt gcc gcg gcg ggg gga gtg ctg ggc ggc tgg      293
Ala Val Val Thr Leu Gly Ala Ala Ala Gly Gly Val Leu Gly Gly Trp
75                      80                      85

ctg gtg gac cgc gcc ggg cgc aag ctg agc ctc ttg ctg tgc tcc gtg      341
Leu Val Asp Arg Ala Gly Arg Lys Leu Ser Leu Leu Leu Cys Ser Val
90                      95                      100                      105

ccc ttc gtg gcc ggc ttt gcc gtc atc acc gcg gcc cag gac gtg tgg      389
Pro Phe Val Ala Gly Phe Ala Val Ile Thr Ala Ala Gln Asp Val Trp
                      110                      115                      120

atg ctg ctg ggg ggc cgc ctc ctc acc ggc ctg gcc tgc ggt gtt gcc      437
Met Leu Leu Gly Gly Arg Leu Leu Thr Gly Leu Ala Cys Gly Val Ala
125                      130                      135

tcc cta gtg gcc ccg gtc tac atc tcc gaa atc gcc tac cca gca gtc      485
Ser Leu Val Ala Pro Val Tyr Ile Ser Glu Ile Ala Tyr Pro Ala Val
140                      145                      150

cgg ggg ttg ctc ggc tcc tgt gtg cag cta atg gtc gtc gtc ggc atc      533
Arg Gly Leu Leu Gly Ser Cys Val Gln Leu Met Val Val Val Gly Ile
155                      160                      165

ctc ctg gcc tac ctg gca ggc tgg gtg ctg gag tgg cgc tgg ctg gct      581
Leu Leu Ala Tyr Leu Ala Gly Trp Val Leu Glu Trp Arg Trp Leu Ala
170                      175                      180                      185

gtg ctg ggc tgc gtg ccc ccc tcc ctc atg ctg ctt ctc atg tgc ttc      629
Val Leu Gly Cys Val Pro Pro Ser Leu Met Leu Leu Leu Met Cys Phe
190                      195                      200

atg ccc gag acc ccg cgc ttc ctg ctg act cag cac agg cgc cag gag      677
Met Pro Glu Thr Pro Arg Phe Leu Thr Gln His Arg Arg Gln Glu
205                      210                      215

gcc atg gcc gcc ctg cgg ttc ctg tgg ggc tcc gag cag ggc tgg gaa      725
Ala Met Ala Ala Leu Arg Phe Leu Trp Gly Ser Glu Gln Gly Trp Glu

```

220	225	230	
gac ccc ccc atc ggg gct gag cag agc ttt cac ctg gcc ctg ctg cgg	773		
Asp Pro Pro Ile Gly Ala Glu Gln Ser Phe His Leu Ala Leu Leu Arg			
235 240 245			
cag ccc ggc atc tac aag ccc ttc atc atc ggc gtc tcc ctg atg gcc	821		
Gln Pro Gly Ile Tyr Lys Pro Phe Ile Ile Gly Val Ser Leu Met Ala			
250 255 260 265			
ttc cag cag ctg tgc ggg gtc aac gcc gtc atg ttc tat gca gag acc	869		
Phe Gln Gln Leu Ser Gly Val Asn Ala Val Met Phe Tyr Ala Glu Thr			
270 275 280			
atc ttt gaa gag gcc aag ttc aag gac agc agc ctg gcc tgc gtc gtc	917		
Ile Phe Glu Glu Ala Lys Phe Lys Asp Ser Ser Leu Ala Ser Val Val			
285 290 295			
gtg ggt gtc atc cag gtg ctg ttc aca gct gtg gcg gct ctc atc atg	965		
Val Gly Val Ile Gln Val Leu Phe Thr Ala Val Ala Ala Leu Ile Met			
300 305 310			
gac aga gca ggg cgg agg ctg ctc ctg gtc ttg tca ggt gtg gtc atg	1013		
Asp Arg Ala Gly Arg Arg Leu Leu Leu Val Leu Ser Gly Val Val Met			
315 320 325			
gtg ttc agc acg agt gcc ttc ggc gcc tac ttc aag ctg acc cag ggt	1061		
Val Phe Ser Thr Ser Ala Phe Gly Ala Tyr Phe Lys Leu Thr Gln Gly			
330 335 340 345			
ggc cct ggc aac tcc tgc cac gtg gcc atc tgc gcg cct gtc tct gca	1109		
Gly Pro Gly Asn Ser Ser His Val Ala Ile Ser Ala Pro Val Ser Ala			
350 355 360			
cag cct gtt gat gcc agc gtg ggg ctg gcc tgg ctg gcc gtg ggc agc	1157		
Gln Pro Val Asp Ala Ser Val Gly Leu Ala Trp Leu Ala Val Gly Ser			
365 370 375			
atg tgc ctc ttc atc gcc ggc ttt gcg gtg ggc tgg ggg ccc atc ccc	1205		
Met Cys Leu Phe Ile Ala Gly Phe Ala Val Gly Trp Gly Pro Ile Pro			
380 385 390			
tgg ctc ctc atg tca gag atc ttc cct ctg cat gtc aag ggc gtg gcg	1253		
Trp Leu Leu Met Ser Glu Ile Phe Pro Leu His Val Lys Gly Val Ala			
395 400 405			
aca ggc atc tgc gtc ctc acc aac tgg ctc atg gcc ttt ctc gtg acc	1301		
Thr Gly Ile Cys Val Leu Thr Asn Trp Leu Met Ala Phe Leu Val Thr			
410 415 420 425			
aag gag ttc agc agc ctc atg gag gtc ctc agg ccc tat gga gcc ttc	1349		
Lys Glu Phe Ser Ser Leu Met Glu Val Leu Arg Pro Tyr Gly Ala Phe			
430 435 440			
tgg ctt gcc tcc gct ttc tgc atc ttc agt gtc ctt ttc act ttt tcc	1397		
Trp Leu Ala Ser Ala Phe Cys Ile Phe Ser Val Leu Phe Thr Phe Ser			
445 450 455			
tgt gtc cct gaa act aaa gga aag act ctg gaa caa atc aca gcc cat	1445		
Cys Val Pro Glu Thr Lys Gly Lys Thr Leu Glu Gln Ile Thr Ala His			
460 465 470			
ttt gag ggg cga tga cagccactca ctaggggatg gagcaagcct gtgactccaa	1500		

Phe Glu Gly Arg
475

gctgggcc

1508

<210> 48
<211> 477
<212> PRT
<213> GLUT8

<400> 48

Met Thr Pro Glu Asp Pro Glu Glu Thr Gln Pro Leu Leu Gly Pro Pro
1 5 10 15

Gly Gly Ser Ala Pro Arg Gly Arg Arg Val Phe Leu Ala Ala Phe Ala
20 25 30

Ala Ala Leu Gly Pro Leu Ser Phe Gly Phe Ala Leu Gly Tyr Ser Ser
35 40 45

Pro Ala Ile Pro Ser Leu Gln Arg Ala Ala Pro Pro Ala Pro Arg Leu
50 55 60

Asp Asp Ala Ala Ala Ser Trp Phe Gly Ala Val Val Thr Leu Gly Ala
65 70 75 80

Ala Ala Gly Gly Val Leu Gly Gly Trp Leu Val Asp Arg Ala Gly Arg
85 90 95

Lys Leu Ser Leu Leu Leu Cys Ser Val Pro Phe Val Ala Gly Phe Ala
100 105 110

Val Ile Thr Ala Ala Gln Asp Val Trp Met Leu Leu Gly Gly Arg Leu
115 120 125

Leu Thr Gly Leu Ala Cys Gly Val Ala Ser Leu Val Ala Pro Val Tyr
130 135 140

Ile Ser Glu Ile Ala Tyr Pro Ala Val Arg Gly Leu Leu Gly Ser Cys
145 150 155 160

Val Gln Leu Met Val Val Val Gly Ile Leu Leu Ala Tyr Leu Ala Gly
165 170 175

Trp Val Leu Glu Trp Arg Trp Leu Ala Val Leu Gly Cys Val Pro Pro
180 185 190

Ser Leu Met Leu Leu Leu Met Cys Phe Met Pro Glu Thr Pro Arg Phe
195 200 205

Leu Leu Thr Gln His Arg Arg Gln Glu Ala Met Ala Ala Leu Arg Phe
 210 215 220

Leu Trp Gly Ser Glu Gln Gly Trp Glu Asp Pro Pro Ile Gly Ala Glu
 225 230 235 240

Gln Ser Phe His Leu Ala Leu Leu Arg Gln Pro Gly Ile Tyr Lys Pro
 245 250 255

Phe Ile Ile Gly Val Ser Leu Met Ala Phe Gln Gln Leu Ser Gly Val
 260 265 270

Asn Ala Val Met Phe Tyr Ala Glu Thr Ile Phe Glu Glu Ala Lys Phe
 275 280 285

Lys Asp Ser Ser Leu Ala Ser Val Val Val Gly Val Ile Gln Val Leu
 290 295 300

Phe Thr Ala Val Ala Ala Leu Ile Met Asp Arg Ala Gly Arg Arg Leu
 305 310 315 320

Leu Leu Val Leu Ser Gly Val Val Met Val Phe Ser Thr Ser Ala Phe
 325 330 335

Gly Ala Tyr Phe Lys Leu Thr Gln Gly Gly Pro Gly Asn Ser Ser His
 340 345 350

Val Ala Ile Ser Ala Pro Val Ser Ala Gln Pro Val Asp Ala Ser Val
 355 360 365

Gly Leu Ala Trp Leu Ala Val Gly Ser Met Cys Leu Phe Ile Ala Gly
 370 375 380

Phe Ala Val Gly Trp Gly Pro Ile Pro Trp Leu Leu Met Ser Glu Ile
 385 390 395 400

Phe Pro Leu His Val Lys Gly Val Ala Thr Gly Ile Cys Val Leu Thr
 405 410 415

Asn Trp Leu Met Ala Phe Leu Val Thr Lys Glu Phe Ser Ser Leu Met
 420 425 430

Glu Val Leu Arg Pro Tyr Gly Ala Phe Trp Leu Ala Ser Ala Phe Cys
 435 440 445

Ile Phe Ser Val Leu Phe Thr Phe Ser Cys Val Pro Glu Thr Lys Gly

450 455 460

Lys Thr Leu Glu Gln Ile Thr Ala His Phe Glu Gly Arg
 465 470 475

<210> 49
 <211> 1863
 <212> DNA
 <213> GLUT9

<220>
 <221> CDS
 <222> (55)..(1677)
 <223>

<400> 49
 cttggcagag tctggggtcc ctggactgag ccatcagctg ggtcactgag accc atg 57
 Met
 1

gca agg aaa caa aat agg aat tcc aag gaa ctg ggc cta gtt ccc ctc 105
 Ala Arg Lys Gln Asn Arg Asn Ser Lys Glu Leu Gly Leu Val Pro Leu
 5 10 15

aca gat gac acc agc cac gcc agg cct cca ggg cca ggg agg gca ctg 153
 Thr Asp Asp Thr Ser His Ala Arg Pro Pro Gly Pro Gly Arg Ala Leu
 20 25 30

ctg gag tgt gac cac ctg agg agt ggg gtg cca ggt gga agg aga aga 201
 Leu Glu Cys Asp His Leu Arg Ser Gly Val Pro Gly Gly Arg Arg Arg
 35 40 45

aag gac tgg tcc tgc tgc ctc ctc gtg gcc tcc ctc gcg ggc gcc ttc 249
 Lys Asp Trp Ser Cys Ser Leu Leu Val Ala Ser Leu Ala Gly Ala Phe
 50 55 60 65

ggc tcc tcc ttc ctc tac ggc tac aac ctg tgc gtg gtg aat gcc ccc 297
 Gly Ser Ser Phe Leu Tyr Gly Tyr Asn Leu Ser Val Val Asn Ala Pro
 70 75 80

acc ccg tac atc aag gcc ttt tac aat gag tca tgg gaa aga agg cat 345
 Thr Pro Tyr Ile Lys Ala Phe Tyr Asn Glu Ser Trp Glu Arg Arg His
 85 90 95

gga cgt cca ata gac cca gac act ctg act ctg ctc tgg tct gtg act 393
 Gly Arg Pro Ile Asp Pro Asp Thr Leu Thr Leu Leu Trp Ser Val Thr
 100 105 110

gtg tcc ata ttc gcc atc ggt gga ctt gtg ggg aca tta att gtg aag 441
 Val Ser Ile Phe Ala Ile Gly Gly Leu Val Gly Thr Leu Ile Val Lys
 115 120 125

atg att gga aag gtt ctt ggg agg aag cac act ttg ctg gcc aat aat 489
 Met Ile Gly Lys Val Leu Gly Arg Lys His Thr Leu Leu Ala Asn Asn
 130 135 140 145

ggg ttt gca att tct gct gca ttg ctg atg gcc tgc tgc ctc cag gca 537
 Gly Phe Ala Ile Ser Ala Ala Leu Leu Met Ala Cys Ser Leu Gln Ala
 150 155 160

gga gcc ttt gaa atg ctc atc gtg gga cgc ttc atc atg ggc ata gat 585

Gly	Ala	Phe	Glu	Met	Leu	Ile	Val	Gly	Arg	Phe	Ile	Met	Gly	Ile	Asp	
			165					170					175			
gga	ggc	gtc	gcc	ctc	agt	gtg	ctc	ccc	atg	tac	ctc	agt	gag	atc	tca	633
Gly	Gly	Val	Ala	Leu	Ser	Val	Leu	Pro	Met	Tyr	Leu	Ser	Glu	Ile	Ser	
		180					185					190				
ccc	aag	gag	atc	cgt	ggc	tct	ctg	ggg	cag	gtg	act	gcc	atc	ttt	atc	681
Pro	Lys	Glu	Ile	Arg	Gly	Ser	Leu	Gly	Gln	Val	Thr	Ala	Ile	Phe	Ile	
	195					200					205					
tgc	att	ggc	gtg	ttc	act	ggg	cag	ctt	ctg	ggc	ctg	ccc	gag	ctg	ctg	729
Cys	Ile	Gly	Val	Phe	Thr	Gly	Gln	Leu	Leu	Gly	Leu	Pro	Glu	Leu	Leu	
210					215					220					225	
gga	aag	gag	agt	acc	tgg	cca	tac	ctg	ttt	gga	gtg	att	gtg	gtc	cct	777
Gly	Lys	Glu	Ser	Thr	Trp	Pro	Tyr	Leu	Phe	Gly	Val	Ile	Val	Val	Pro	
				230					235					240		
gcc	gtt	gtc	cag	ctg	ctg	agc	ctt	ccc	ttt	ctc	ccg	gac	agc	cca	cgc	825
Ala	Val	Val	Gln	Leu	Leu	Ser	Leu	Pro	Phe	Leu	Pro	Asp	Ser	Pro	Arg	
			245					250					255			
tac	ctg	ctc	ttg	gag	aag	cac	aac	gag	gca	aga	gct	gtg	aaa	gcc	ttc	873
Tyr	Leu	Leu	Leu	Glu	Lys	His	Asn	Glu	Ala	Arg	Ala	Val	Lys	Ala	Phe	
		260					265					270				
caa	acg	ttc	ttg	ggt	aaa	gca	gac	gtt	tcc	caa	gag	gta	gag	gag	gtc	921
Gln	Thr	Phe	Leu	Gly	Lys	Ala	Asp	Val	Ser	Gln	Glu	Val	Glu	Glu	Val	
	275					280					285					
ctg	gct	gag	agc	cgc	gtg	cag	agg	agc	atc	cgc	ctg	gtg	tcc	gtg	ctg	969
Leu	Ala	Glu	Ser	Arg	Val	Gln	Arg	Ser	Ile	Arg	Leu	Val	Ser	Val	Leu	
290					295					300					305	
gag	ctg	ctg	aga	gct	ccc	tac	gtc	cgc	tgg	cag	gtg	gtc	acc	gtg	att	1017
Glu	Leu	Leu	Arg	Ala	Pro	Tyr	Val	Arg	Trp	Gln	Val	Val	Thr	Val	Ile	
				310					315					320		
gtc	acc	atg	gcc	tgc	tac	cag	ctc	tgt	ggc	ctc	aat	gca	att	tgg	ttc	1065
Val	Thr	Met	Ala	Cys	Tyr	Gln	Leu	Cys	Gly	Leu	Asn	Ala	Ile	Trp	Phe	
			325					330					335			
tat	acc	aac	agc	atc	ttt	gga	aaa	gct	ggg	atc	cct	ctg	gca	aag	atc	1113
Tyr	Thr	Asn	Ser	Ile	Phe	Gly	Lys	Ala	Gly	Ile	Pro	Leu	Ala	Lys	Ile	
		340					345					350				
cca	tac	gtc	acc	ttg	agt	aca	ggg	ggc	atc	gag	act	ttg	gct	gcc	gtc	1161
Pro	Tyr	Val	Thr	Leu	Ser	Thr	Gly	Gly	Ile	Glu	Thr	Leu	Ala	Ala	Val	
		355				360					365					
ttc	tct	ggt	ttg	gtc	att	gag	cac	ctg	gga	cgg	aga	ccc	ctc	ctc	att	1209
Phe	Ser	Gly	Leu	Val	Ile	Glu	His	Leu	Gly	Arg	Arg	Pro	Leu	Leu	Ile	
370					375					380					385	
ggt	ggc	ttt	ggg	ctc	atg	ggc	ctc	ttc	ttt	ggg	acc	ctc	acc	atc	acg	1257
Gly	Gly	Phe	Gly	Leu	Met	Gly	Leu	Phe	Phe	Gly	Thr	Leu	Thr	Ile	Thr	
				390					395					400		
ctg	acc	ctg	cag	gac	cac	gcc	ccc	tgg	gtc	ccc	tac	ctg	agt	atc	gtg	1305
Leu	Thr	Leu	Gln	Asp	His	Ala	Pro	Trp	Val	Pro	Tyr	Leu	Ser	Ile	Val	
			405					410					415			

ggc att ctg gcc atc atc gcc tct ttc tgc agt ggg cca ggt ggc atc 1353
 Gly Ile Leu Ala Ile Ile Ala Ser Phe Cys Ser Gly Pro Gly Gly Ile
 420 425 430

ccg ttc atc ttg act ggt gag ttc ttc cag caa tct cag cgg ccg gct 1401
 Pro Phe Ile Leu Thr Gly Glu Phe Phe Gln Gln Ser Gln Arg Pro Ala
 435 440 445

gcc ttc atc att gca ggc acc gtc aac tgg ctc tcc aac ttt gct gtt 1449
 Ala Phe Ile Ile Ala Gly Thr Val Asn Trp Leu Ser Asn Phe Ala Val
 450 455 460 465

ggg ctc ctc ttc cca ttc att cag aaa agt ctg gac acc tac tgt ttc 1497
 Gly Leu Leu Phe Pro Phe Ile Gln Lys Ser Leu Asp Thr Tyr Cys Phe
 470 475 480

cta gtc ttt gct aca att tgt atc aca ggt gct atc tac ctg tat ttt 1545
 Leu Val Phe Ala Thr Ile Cys Ile Thr Gly Ala Ile Tyr Leu Tyr Phe
 485 490 495

gtg ctg cct gag acc aaa aac aga acc tat gca gaa atc agc cag gca 1593
 Val Leu Pro Glu Thr Lys Asn Arg Thr Tyr Ala Glu Ile Ser Gln Ala
 500 505 510

ttt tcc aaa agg aac aaa gca tac cca cca gaa gag aaa atc gac tca 1641
 Phe Ser Lys Arg Asn Lys Ala Tyr Pro Pro Glu Glu Lys Ile Asp Ser
 515 520 525

gct gtc act gat ggt aag ata aat gga agg cct taa caagtttcct 1687
 Ala Val Thr Asp Gly Lys Ile Asn Gly Arg Pro
 530 535 540

cctccacggt ggacaattat gtcaaaaaca ggattgtcta catggatgat ctcaacttttc 1747

aggaaactta aaatttacc attattggga agcttaaagt aattgaagct atgcaagtct 1807

tttatattat taaatattta aaagtaaacc tgtactaatc taaaaaaaaa aaaaaa 1863

<210> 50
 <211> 540
 <212> PRT
 <213> GLUT9

<400> 50

Met Ala Arg Lys Gln Asn Arg Asn Ser Lys Glu Leu Gly Leu Val Pro
 1 5 10 15

Leu Thr Asp Asp Thr Ser His Ala Arg Pro Pro Gly Pro Gly Arg Ala
 20 25 30

Leu Leu Glu Cys Asp His Leu Arg Ser Gly Val Pro Gly Gly Arg Arg
 35 40 45

Arg Lys Asp Trp Ser Cys Ser Leu Leu Val Ala Ser Leu Ala Gly Ala
 50 55 60

Phe Gly Ser Ser Phe Leu Tyr Gly Tyr Asn Leu Ser Val Val Asn Ala

97

65		70		75		80
Pro Thr Pro Tyr	Ile Lys Ala Phe Tyr Asn Glu Ser Trp Glu Arg Arg					
	85			90		95
His Gly Arg Pro	Ile Asp Pro Asp Thr Leu Thr Leu Leu Trp Ser Val					
	100		105		110	
Thr Val Ser Ile Phe Ala	Ile Gly Gly Leu Val Gly Thr Leu Ile Val					
	115		120		125	
Lys Met Ile Gly Lys Val	Leu Gly Arg Lys His Thr Leu Leu Ala Asn					
	130		135		140	
Asn Gly Phe Ala Ile Ser Ala Ala	Leu Leu Met Ala Cys Ser Leu Gln					
145		150		155		160
Ala Gly Ala Phe Glu Met Leu Ile Val	Gly Arg Phe Ile Met Gly Ile					
	165		170		175	
Asp Gly Gly Val Ala Leu Ser Val	Leu Pro Met Tyr Leu Ser Glu Ile					
	180		185		190	
Ser Pro Lys Glu Ile Arg Gly Ser Leu Gly Gln Val Thr Ala Ile Phe						
	195		200		205	
Ile Cys Ile Gly Val Phe Thr Gly Gln Leu Leu Gly Leu Pro Glu Leu						
	210		215		220	
Leu Gly Lys Glu Ser Thr Trp Pro Tyr Leu Phe Gly Val Ile Val Val						
225		230		235		240
Pro Ala Val Val Gln Leu Leu Ser Leu Pro Phe Leu Pro Asp Ser Pro						
	245		250		255	
Arg Tyr Leu Leu Leu Glu Lys His Asn Glu Ala Arg Ala Val Lys Ala						
	260		265		270	
Phe Gln Thr Phe Leu Gly Lys Ala Asp Val Ser Gln Glu Val Glu Glu						
	275		280		285	
Val Leu Ala Glu Ser Arg Val Gln Arg Ser Ile Arg Leu Val Ser Val						
	290		295		300	
Leu Glu Leu Leu Arg Ala Pro Tyr Val Arg Trp Gln Val Val Thr Val						
305		310		315		320

Ile Val Thr Met Ala Cys Tyr Gln Leu Cys Gly Leu Asn Ala Ile Trp
325 330 335

Phe Tyr Thr Asn Ser Ile Phe Gly Lys Ala Gly Ile Pro Leu Ala Lys
340 345 350

Ile Pro Tyr Val Thr Leu Ser Thr Gly Gly Ile Glu Thr Leu Ala Ala
355 360 365

Val Phe Ser Gly Leu Val Ile Glu His Leu Gly Arg Arg Pro Leu Leu
370 375 380

Ile Gly Gly Phe Gly Leu Met Gly Leu Phe Phe Gly Thr Leu Thr Ile
385 390 395 400

Thr Leu Thr Leu Gln Asp His Ala Pro Trp Val Pro Tyr Leu Ser Ile
405 410 415

Val Gly Ile Leu Ala Ile Ile Ala Ser Phe Cys Ser Gly Pro Gly Gly
420 425 430

Ile Pro Phe Ile Leu Thr Gly Glu Phe Phe Gln Gln Ser Gln Arg Pro
435 440 445

Ala Ala Phe Ile Ile Ala Gly Thr Val Asn Trp Leu Ser Asn Phe Ala
450 455 460

Val	Gly	Leu	Leu	Phe	Pro	Phe	Ile	Gln	Lys	Ser	Leu	Asp	Thr	Tyr	Cys
465					470					475					480

Phe Leu Val Phe Ala Thr Ile Cys Ile Thr Gly Ala Ile Tyr Leu Tyr
485 490 495

Phe Val Leu Pro Glu Thr Lys Asn Arg Thr Tyr Ala Glu Ile Ser Gln
500 505 510

Ala Phe Ser Lys Arg Asn Lys Ala Tyr Pro Pro Glu Glu Lys Ile Asp
515 520 525

Ser Ala Val Thr Asp Gly Lys Ile Asn Gly Arg Pro
530 535 540

<210>	51
<211>	4167
<212>	DNA
<213>	GLUT10

<220>
<221> CDS

<222> (53)..(1678)

<223>

<400> 51

```

atgcgcgccc ggcccctcag cgccccagc acgccgccga gtcccgcctcg cc atg ggc      58
                                     Met Gly
                                     1

cac tcc cca cct gtc ctg cct ttg tgt gcc tct gtg tct ttg ctg ggt      106
His Ser Pro Pro Val Leu Pro Leu Cys Ala Ser Val Ser Leu Leu Gly
      5                                10                                15

ggc ctg acc ttt ggt tat gaa ctg gca gtc ata tca ggt gcc ctg ctg      154
Gly Leu Thr Phe Gly Tyr Glu Leu Ala Val Ile Ser Gly Ala Leu Leu
      20                                25                                30

cca ctg cag ctt gac ttt ggg cta agc tgc ttg gag cag gag ttc ctg      202
Pro Leu Gln Leu Asp Phe Gly Leu Ser Cys Leu Glu Gln Glu Phe Leu
      35                                40                                45                                50

gtg ggc agc ctg ctc ctg ggg gct ctc ctc gcc tcc ctg gtt ggt ggc      250
Val Gly Ser Leu Leu Leu Gly Ala Leu Leu Ala Ser Leu Val Gly Gly
      55                                60                                65

ttc ctc att gac tgc tat ggc agg aag caa gcc atc ctc ggg agc aac      298
Phe Leu Ile Asp Cys Tyr Gly Arg Lys Gln Ala Ile Leu Gly Ser Asn
      70                                75                                80

ttg gtg ctg ctg gca ggc agc ctg acc ctg ggc ctg gct ggt tcc ctg      346
Leu Val Leu Leu Ala Gly Ser Leu Thr Leu Gly Leu Ala Gly Ser Leu
      85                                90                                95

gcc tgg ctg gtc ctg ggc cgc gct gtg gtt ggc ttc gcc att tcc ctc      394
Ala Trp Leu Val Leu Gly Arg Ala Val Val Gly Phe Ala Ile Ser Leu
      100                                105                                110

tcc tcc atg gct tgc tgt atc tac gtg tca gag ctg gtg ggg cca cgg      442
Ser Ser Met Ala Cys Cys Ile Tyr Val Ser Glu Leu Val Gly Pro Arg
      115                                120                                125                                130

cag cgg gga gtg ctg gtc tcc ctc tat gag gca ggc atc acc gtg ggc      490
Gln Arg Gly Val Leu Val Ser Leu Tyr Glu Ala Gly Ile Thr Val Gly
      135                                140                                145

atc ctg ctc tcc tat gcc ctc aac tat gca ctg gct ggt acc ccc tgg      538
Ile Leu Leu Ser Tyr Ala Leu Asn Tyr Ala Leu Ala Gly Thr Pro Trp
      150                                155                                160

gga tgg agg cac atg ttc ggc tgg gcc act gca cct gct gtc ctg caa      586
Gly Trp Arg His Met Phe Gly Trp Ala Thr Ala Pro Ala Val Leu Gln
      165                                170                                175

tcc ctc agc ctc ctc ttc ctc cct gct ggt aca gat gag act gca aca      634
Ser Leu Ser Leu Leu Phe Leu Pro Ala Gly Thr Asp Glu Thr Ala Thr
      180                                185                                190

cac aag gac ctc atc cca ctc cag gga ggt gag gcc ccc aag ctg ggc      682
His Lys Asp Leu Ile Pro Leu Gln Gly Gly Glu Ala Pro Lys Leu Gly
      195                                200                                205                                210

ccg ggg agg cca cgg tac tcc ttt ctg gac ctc ttc agg gca cgc gat      730
Pro Gly Arg Pro Arg Tyr Ser Phe Leu Asp Leu Phe Arg Ala Arg Asp
      215                                220                                225

```

aac atg cga ggc cgg acc aca gtg ggc ctg ggg ctg gtg ctc ttc cag	778
Asn Met Arg Gly Arg Thr Thr Val Gly Leu Gly Leu Val Leu Phe Gln	
230 235 240	
caa cta aca ggg cag ccc aac gtg ctg tgc tat gcc tcc acc atc ttc	826
Gln Leu Thr Gly Gln Pro Asn Val Leu Cys Tyr Ala Ser Thr Ile Phe	
245 250 255	
agc tcc gtt ggt ttc cat ggg gga tcc tca gcc gtg ctg gcc tct gtg	874
Ser Ser Val Gly Phe His Gly Gly Ser Ser Ala Val Leu Ala Ser Val	
260 265 270	
ggg ctt ggc gca gtg aag gtg gca gct acc ctg acc gcc atg ggg ctg	922
Gly Leu Gly Ala Val Lys Val Ala Ala Thr Leu Thr Ala Met Gly Leu	
275 280 285 290	
gtg gac cgt gca ggc cgc agg gct ctg ttg cta gct ggc tgt gcc ctc	970
Val Asp Arg Ala Gly Arg Arg Ala Leu Leu Ala Gly Cys Ala Leu	
295 300 305	
atg gcc ctg tcc gtc agt ggc ata ggc ctc gtc agc ttt gcc gtg ccc	1018
Met Ala Leu Ser Val Ser Gly Ile Gly Leu Val Ser Phe Ala Val Pro	
310 315 320	
atg gac tca ggc cca agc tgt ctg gct gtg ccc aat gcc acc ggg cag	1066
Met Asp Ser Gly Pro Ser Cys Leu Ala Val Pro Asn Ala Thr Gly Gln	
325 330 335	
aca ggc ctc cct gga gac tct ggc ctg ctg cag gac tcc tct cta cct	1114
Thr Gly Leu Pro Gly Asp Ser Gly Leu Leu Gln Asp Ser Ser Leu Pro	
340 345 350	
ccc att cca agg acc aat gag gac caa agg gag cca atc ttg tcc act	1162
Pro Ile Pro Arg Thr Asn Glu Asp Gln Arg Glu Pro Ile Leu Ser Thr	
355 360 365 370	
gct aag aaa acc aag ccc cat ccc aga tct gga gac ccc tca gcc cct	1210
Ala Lys Lys Thr Lys Pro His Pro Arg Ser Gly Asp Pro Ser Ala Pro	
375 380 385	
cct cgg ctg gcc ctg agc tct gcc ctc cct ggg ccc cct ctg ccc gct	1258
Pro Arg Leu Ala Leu Ser Ser Ala Leu Pro Gly Pro Pro Leu Pro Ala	
390 395 400	
cgg ggg cat gca ctg ctg cgc tgg acc gca ctg ctg tgc ctg atg gtc	1306
Arg Gly His Ala Leu Leu Arg Trp Thr Ala Leu Leu Cys Leu Met Val	
405 410 415	
ttt gtc agt gcc ttc tcc ttt ggg ttt ggg cca gtg acc tgg ctt gtc	1354
Phe Val Ser Ala Phe Ser Phe Gly Phe Gly Pro Val Thr Trp Leu Val	
420 425 430	
ctc agc gag atc tac cct gtg gag ata cga gga aga gcc ttc gcc ttc	1402
Leu Ser Glu Ile Tyr Pro Val Glu Ile Arg Gly Arg Ala Phe Ala Phe	
435 440 445 450	
tgc aac agc ttc aac tgg gcg gcc aac ctc ttc atc agc ctc tcc ttc	1450
Cys Asn Ser Phe Asn Trp Ala Ala Asn Leu Phe Ile Ser Leu Ser Phe	
455 460 465	
ctc gat ctc att ggc acc atc ggc ttg tcc tgg acc ttc ctg ctc tac	1498
Leu Asp Leu Ile Gly Thr Ile Gly Leu Ser Trp Thr Phe Leu Leu Tyr	

470	475	480	
gga ctg acc gct gtc ctc ggc ctg ggc ttc atc tat tta ttt gtt cct			1546
Gly Leu Thr Ala Val Leu Gly Leu Gly Phe Ile Tyr Leu Phe Val Pro			
485	490	495	
gaa aca aaa ggc cag tcg ttg gca gag ata gac cag cag ttc cag aag			1594
Glu Thr Lys Gly Gln Ser Leu Ala Glu Ile Asp Gln Gln Phe Gln Lys			
500	505	510	
aga cgg ttc acc ctg agc ttt ggc cac agg cag aac tcc act ggc atc			1642
Arg Arg Phe Thr Leu Ser Phe Gly His Arg Gln Asn Ser Thr Gly Ile			
515	520	525	530
ccg tac agc cgc atc gag atc tct gcg gcc tcc tga ggaatccgtc			1688
Pro Tyr Ser Arg Ile Glu Ile Ser Ala Ala Ser			
535	540		
tgccctgga aa ttctggaact gtggcttttg cagaccatct ccagcatcct gcttcctagg			1748
ccccagagca caagttccag ctgggtctttt gggagtggcc cctgccccca aagggtgtct			1808
gcttttgctg gggtaaaaag gatgaaagtc tgagaatgcc caactcttca ttttgagtct			1868
caggccctga aggttcctga ggatctagct tcatgcctca gtttcccat tgacttgcac			1928
atctctgcag tattttataag aagaatattc tatgaagtct ttgttgcacc atggactttt			1988
ctcaaagaat ctcaagggtta ccaatcctgg caggaagtct ctcccgatat caccctataa			2048
tccaaatgag gatattcatct tttctaatct cttttttcaa ctggctggga cattttcgga			2108
agggggaagt ctcttttttt actcttatca tttttttttt ttgaggtgga gtctcattct			2168
gttgcccagg ctggcctgat cttggctcac tgcaacctcc acctcctgag ttcaagcgat			2228
tcttgctgct cagcctccta agcagctggg actacaggcg catgcaacca taccagcta			2288
atttattttt agcagagatg gggtttctact gtgttgcca ggctggctcg gaactcctga			2348
gctcaagtga tccaccacc tcagcctccc agagtgctag gattacaggc cttttgactc			2408
ttttatctga gttttattga cccctcta at tctcttacc agaataattta tccttcacca			2468
gcaactctga ctctttgacg ggaggcctca gttctagtcc ttgggtctgct ggtgtcattg			2528
ctgtaggaat gaccacgggc ctcatgttcc ccatttgtat aatgggaagc ctgtaccagg			2588
tcattcttaa gatttctcct gactccagtg agctggaatt ctaaagtctg gtctaggagc			2648
tgtctccagg atgggtcagg atggctttgc ggaaaggaga tgggtttgga ggccaacaaa			2708
cctgcttgct aatattgcct ttgcctcttg gcagcccttg aacttgagta aataacaact			2768
ccctgaacct cagtttcctc atctgcagaa tggggataat tatgtcccag ggtatatatt			2828
agaccctgtt tcctttcagg agggccccca gctggtccag ggcctgggaa atttctactt			2888
atcctcatta cccaggctcc tcctttggac cctgtaaagg gtcagggtga atcagatggg			2948
ggactgagca agtagctatg actgcagatc atgtaaggaa gggactgaca agaagctccc			3008
agatgctggg gagaatgaag agctaaaata gatcctaggt gctggatgct ttgtcatoca			3068

tgcgtgcaca tatgggtgct ggcagagccc ccaaggactc tggcctctcg agttctccta 3128
 tcttctccat tctagatgct tcccttgat cagtgatgt gctggagctg gctttgccaa 3188
 gcttgtgaga gctggttgct acattttcag gatttttaca agttggtaaa cacagccatt 3248
 ataaaaaatt aaatgattta aatttataat taagtaaatt acattaaaac aaaaaatta 3308
 tactcaaaat tcattactta attttactac ctgttactat tatctgtgct tttgaggcta 3368
 tttctacata gtaactctta tggagaccta ggggagacac cgcgcatctc ttcttgattc 3428
 cccactcaat gacatcatgt tagtctttgg ttgcttaact ggctgtgggg agtggttttg 3488
 tatcacaaag attagagagg actacacatc agggcttgat ttattgtttg ttgattttct 3548
 agacttcaga acatgctgga taaaatgtca gtaatgcaa ttaaaacttta aagtatgtct 3608
 tgttttagtc caatacatgg tgtatagcac caaaaaatgg agggattatt cttccagtag 3668
 ttgaacactg tcatccgttt cagctgacag ctgctcaaat catttaagaa ggagttctga 3728
 cattcatttt cattgtttta cttttgtctt cctcactagt gtaaacaaaa atttcaacca 3788
 gcattcatgc cgaacctata ccattcttc agtgcctagc tgtacagtta tcagggattt 3848
 ttattttag tctaattttg tcaaatcatg gccaaatcgc agtgatagtt gactttggat 3908
 acaaggtttg gcaaaaaaaaa aaatattaac aaaatattct gtaagaatca attgtctata 3968
 tggaatttag gataaagaat atttacaata aagaatattt acaataaaga gtttattatt 4028
 atttgaagt tgtgtgcaac aacataccc tttatctctg taaaatttat acacacaaaa 4088
 attaacaaaa gattctgtaa gaattaattg gctatatgga atttaggata gaatatttac 4148
 aataaagagt atttacaat 4167

<210> 52
 <211> 541
 <212> PRT
 <213> GLUT10

<400> 52

Met Gly His Ser Pro Pro Val Leu Pro Leu Cys Ala Ser Val Ser Leu
 1 5 10 15

Leu Gly Gly Leu Thr Phe Gly Tyr Glu Leu Ala Val Ile Ser Gly Ala
 20 25 30

Leu Leu Pro Leu Gln Leu Asp Phe Gly Leu Ser Cys Leu Glu Gln Glu
 35 40 45

Phe Leu Val Gly Ser Leu Leu Leu Gly Ala Leu Leu Ala Ser Leu Val
 50 55 60

103

Gly Gly Phe Leu Ile Asp Cys Tyr Gly Arg Lys Gln Ala Ile Leu Gly
 65 70 75 80

Ser Asn Leu Val Leu Leu Ala Gly Ser Leu Thr Leu Gly Leu Ala Gly
 85 90 95

Ser Leu Ala Trp Leu Val Leu Gly Arg Ala Val Val Gly Phe Ala Ile
 100 105 110

Ser Leu Ser Ser Met Ala Cys Cys Ile Tyr Val Ser Glu Leu Val Gly
 115 120 125

Pro Arg Gln Arg Gly Val Leu Val Ser Leu Tyr Glu Ala Gly Ile Thr
 130 135 140

Val Gly Ile Leu Leu Ser Tyr Ala Leu Asn Tyr Ala Leu Ala Gly Thr
 145 150 155 160

Pro Trp Gly Trp Arg His Met Phe Gly Trp Ala Thr Ala Pro Ala Val
 165 170 175

Leu Gln Ser Leu Ser Leu Leu Phe Leu Pro Ala Gly Thr Asp Glu Thr
 180 185 190

Ala Thr His Lys Asp Leu Ile Pro Leu Gln Gly Gly Glu Ala Pro Lys
 195 200 205

Leu Gly Pro Gly Arg Pro Arg Tyr Ser Phe Leu Asp Leu Phe Arg Ala
 210 215 220

Arg Asp Asn Met Arg Gly Arg Thr Thr Val Gly Leu Gly Leu Val Leu
 225 230 235 240

Phe Gln Gln Leu Thr Gly Gln Pro Asn Val Leu Cys Tyr Ala Ser Thr
 245 250 255

Ile Phe Ser Ser Val Gly Phe His Gly Gly Ser Ser Ala Val Leu Ala
 260 265 270

Ser Val Gly Leu Gly Ala Val Lys Val Ala Ala Thr Leu Thr Ala Met
 275 280 285

Gly Leu Val Asp Arg Ala Gly Arg Arg Ala Leu Leu Leu Ala Gly Cys
 290 295 300

Ala Leu Met Ala Leu Ser Val Ser Gly Ile Gly Leu Val Ser Phe Ala
 305 310 315 320

104

Val Pro Met Asp Ser Gly Pro Ser Cys Leu Ala Val Pro Asn Ala Thr
 325 330 335
 Gly Gln Thr Gly Leu Pro Gly Asp Ser Gly Leu Leu Gln Asp Ser Ser
 340 345 350
 Leu Pro Pro Ile Pro Arg Thr Asn Glu Asp Gln Arg Glu Pro Ile Leu
 355 360 365
 Ser Thr Ala Lys Lys Thr Lys Pro His Pro Arg Ser Gly Asp Pro Ser
 370 375 380
 Ala Pro Pro Arg Leu Ala Leu Ser Ser Ala Leu Pro Gly Pro Pro Leu
 385 390 395 400
 Pro Ala Arg Gly His Ala Leu Leu Arg Trp Thr Ala Leu Leu Cys Leu
 405 410 415
 Met Val Phe Val Ser Ala Phe Ser Phe Gly Phe Gly Pro Val Thr Trp
 420 425 430
 Leu Val Leu Ser Glu Ile Tyr Pro Val Glu Ile Arg Gly Arg Ala Phe
 435 440 445
 Ala Phe Cys Asn Ser Phe Asn Trp Ala Ala Asn Leu Phe Ile Ser Leu
 450 455 460
 Ser Phe Leu Asp Leu Ile Gly Thr Ile Gly Leu Ser Trp Thr Phe Leu
 465 470 475 480
 Leu Tyr Gly Leu Thr Ala Val Leu Gly Leu Gly Phe Ile Tyr Leu Phe
 485 490 495
 Val Pro Glu Thr Lys Gly Gln Ser Leu Ala Glu Ile Asp Gln Gln Phe
 500 505 510
 Gln Lys Arg Arg Phe Thr Leu Ser Phe Gly His Arg Gln Asn Ser Thr
 515 520 525
 Gly Ile Pro Tyr Ser Arg Ile Glu Ile Ser Ala Ala Ser
 530 535 540

<210> 53
 <211> 1608
 <212> DNA
 <213> GLUT11

<220>

105

<221> CDS

<222> (100)..(1590)

<223>

<400> 53

```

cggacctgcc tctcacgcaa tggatcccct ggcggcaacc cgagaccggt tctcctaccc      60
gcattccgcc aagtctctcg ctctgccag gacgcacag atg aga gcg ctc cga      114
                                     Met Arg Ala Leu Arg
                                     1           5

aga ctg att cag ggc agg atc ctg ctc ctg acc atc tgc gct gcc ggc      162
Arg Leu Ile Gln Gly Arg Ile Leu Leu Leu Thr Ile Cys Ala Ala Gly
                10                15                20

att ggt ggg act ttt cag ttt ggc tat aac ctc tct atc atc aat gcc      210
Ile Gly Gly Thr Phe Gln Phe Gly Tyr Asn Leu Ser Ile Ile Asn Ala
                25                30                35

ccg acc ttg cac att cag gaa ttc acc aat gag aca tgg cag gcg cgt      258
Pro Thr Leu His Ile Gln Glu Phe Thr Asn Glu Thr Trp Gln Ala Arg
                40                45                50

act gga gag cca ctg ccc gat cac cta gtc ctg ctt atg tgg tcc ctc      306
Thr Gly Glu Pro Leu Pro Asp His Leu Val Leu Leu Met Trp Ser Leu
                55                60                65

atc gtg tct ctg tat ccc ctg gga ggc ctc ttt gga gca ctg ctt gca      354
Ile Val Ser Leu Tyr Pro Leu Gly Gly Leu Phe Gly Ala Leu Leu Ala
                70                75                80                85

ggt ccc ttg gcc atc acg ctg gga agg aag aag tcc ctc ctg gtg aat      402
Gly Pro Leu Ala Ile Thr Leu Gly Arg Lys Lys Ser Leu Leu Val Asn
                90                95                100

aac atc ttt gtg gtg tca gca gca atc ctg ttt gga ttc agc cgc aaa      450
Asn Ile Phe Val Val Ser Ala Ala Ile Leu Phe Gly Phe Ser Arg Lys
                105                110                115

gca ggc tcc ttt gag atg atc atg ctg gga aga ctg ctc gtg gga gtc      498
Ala Gly Ser Phe Glu Met Ile Met Leu Gly Arg Leu Leu Val Gly Val
                120                125                130

aat gca ggt gtg agc atg aac atc cag ccc atg tac ctg ggg gag agc      546
Asn Ala Gly Val Ser Met Asn Ile Gln Pro Met Tyr Leu Gly Glu Ser
                135                140                145

gcc cct aag gag ctc cga gga gct gtg gcc atg agc tca gcc atc ttt      594
Ala Pro Lys Glu Leu Arg Gly Ala Val Ala Met Ser Ser Ala Ile Phe
                150                155                160                165

acg gct ctg ggg atc gtg atg gga cag gtg gtc gga ctc agg gag ctc      642
Thr Ala Leu Gly Ile Val Met Gly Gln Val Val Gly Leu Arg Glu Leu
                170                175                180

cta ggt ggc cct cag gcc tgg ccc ctg ctg ctg gcc agc tgc ctg gtg      690
Leu Gly Gly Pro Gln Ala Trp Pro Leu Leu Leu Ala Ser Cys Leu Val
                185                190                195

ccc ggg gcg ctc cag ctc gcc tcc ctg cct ctg ctc cct gaa agc ccg      738
Pro Gly Ala Leu Gln Leu Ala Ser Leu Pro Leu Leu Pro Glu Ser Pro
                200                205                210

```

cgc tac ctc ctc att gac tgt gga gac acc gag gcc tgc ctg gca gca Arg Tyr Leu Leu Ile Asp Cys Gly Asp Thr Glu Ala Cys Leu Ala Ala 215 220 225	786
cta cgg cgg ctc cgg ggc tcc ggg gac ttg gca ggg gag ctg gag gag Leu Arg Arg Leu Arg Gly Ser Gly Asp Leu Ala Gly Glu Leu Glu Glu 230 235 240 245	834
ctg gag gag gag cgc gct gcc tgc cag ggc tgc cgt gcc cgg cgc cca Leu Glu Glu Glu Arg Ala Ala Cys Gln Gly Cys Arg Ala Arg Arg Pro 250 255 260	882
tgg gag ctg ttc cag cat cgg gcc ctg agg aga cag gtg aca agc ctc Trp Glu Leu Phe Gln His Arg Ala Leu Arg Arg Gln Val Thr Ser Leu 265 270 275	930
gtg gtt ctg ggc agt gcc atg gag ctc tgc ggg aat gac tcg gtg tac Val Val Leu Gly Ser Ala Met Glu Leu Cys Gly Asn Asp Ser Val Tyr 280 285 290	978
gcc tac gcc tcc tcc gtg ttc cgg aag gca gga gtg ccg gaa gcg aag Ala Tyr Ala Ser Ser Val Phe Arg Lys Ala Gly Val Pro Glu Ala Lys 295 300 305	1026
atc cag tac gcg atc atc ggg act ggg agc tgc gag ctg ctc acg gcg Ile Gln Tyr Ala Ile Ile Gly Thr Gly Ser Cys Glu Leu Leu Thr Ala 310 315 320 325	1074
gtt gtt agt tgt gtg gta atc gag agg gtg ggt cgg cgc gtg ctg ctc Val Val Ser Cys Val Val Ile Glu Arg Val Gly Arg Arg Val Leu Leu 330 335 340	1122
atc ggt ggg tac agc ctg atg acc tgc tgg ggg agc atc ttc act gtg Ile Gly Gly Tyr Ser Leu Met Thr Cys Trp Gly Ser Ile Phe Thr Val 345 350 355	1170
gcc ctg tgc ctg cag agc tcc ttc ccc tgg aca ctc tac ctg gcc atg Ala Leu Cys Leu Gln Ser Ser Phe Pro Trp Thr Leu Tyr Leu Ala Met 360 365 370	1218
gcc tgc atc ttt gcc ttc atc ctc agc ttt ggc att ggc cct gcc gga Ala Cys Ile Phe Ala Phe Ile Leu Ser Phe Gly Ile Gly Pro Ala Gly 375 380 385	1266
gtg acg ggg atc ctg gcc aca gag ctg ttt gac cag atg gcc agg cct Val Thr Gly Ile Leu Ala Thr Glu Leu Phe Asp Gln Met Ala Arg Pro 390 395 400 405	1314
gct gcc tgc atg gtc tgc ggg gcg ctc atg tgg atc atg ctc atc ctg Ala Ala Cys Met Val Cys Gly Ala Leu Met Trp Ile Met Leu Ile Leu 410 415 420	1362
gtc ggc ctg gga ttt ccc ttt atc atg gag gcc ttg tcc cac ttc ctc Val Gly Leu Gly Phe Pro Phe Ile Met Glu Ala Leu Ser His Phe Leu 425 430 435	1410
tat gtc cct ttc ctt ggt gtc tgt gtc tgt ggg gcc atc tac act ggc Tyr Val Pro Phe Leu Gly Val Cys Val Cys Gly Ala Ile Tyr Thr Gly 440 445 450	1458
ctg ttc ctt cct gag acc aaa ggc aag acc ttc caa gag atc tcc aag Leu Phe Leu Pro Glu Thr Lys Gly Lys Thr Phe Gln Glu Ile Ser Lys 455 460 465	1506

107

gaa tta cac aga ctc aac ttc ccc agg cgg gcc cag ggc ccc acg tgg 1554
 Glu Leu His Arg Leu Asn Phe Pro Arg Arg Ala Gln Gly Pro Thr Trp
 470 475 480 485

agg agc ctg gag gtt atc cag tca aca gaa ctc tag tcccaaaggg 1600
 Arg Ser Leu Glu Val Ile Gln Ser Thr Glu Leu
 490 495

gtggccag 1608

<210> 54
 <211> 496
 <212> PRT
 <213> GLUT11

<400> 54

Met Arg Ala Leu Arg Arg Leu Ile Gln Gly Arg Ile Leu Leu Leu Thr
 1 5 10 15

Ile Cys Ala Ala Gly Ile Gly Gly Thr Phe Gln Phe Gly Tyr Asn Leu
 20 25 30

Ser Ile Ile Asn Ala Pro Thr Leu His Ile Gln Glu Phe Thr Asn Glu
 35 40 45

Thr Trp Gln Ala Arg Thr Gly Glu Pro Leu Pro Asp His Leu Val Leu
 50 55 60

Leu Met Trp Ser Leu Ile Val Ser Leu Tyr Pro Leu Gly Gly Leu Phe
 65 70 75 80

Gly Ala Leu Leu Ala Gly Pro Leu Ala Ile Thr Leu Gly Arg Lys Lys
 85 90 95

Ser Leu Leu Val Asn Asn Ile Phe Val Val Ser Ala Ala Ile Leu Phe
 100 105 110

Gly Phe Ser Arg Lys Ala Gly Ser Phe Glu Met Ile Met Leu Gly Arg
 115 120 125

Leu Leu Val Gly Val Asn Ala Gly Val Ser Met Asn Ile Gln Pro Met
 130 135 140

Tyr Leu Gly Glu Ser Ala Pro Lys Glu Leu Arg Gly Ala Val Ala Met
 145 150 155 160

Ser Ser Ala Ile Phe Thr Ala Leu Gly Ile Val Met Gly Gln Val Val
 165 170 175

108

Gly Leu Arg Glu Leu Leu Gly Gly Pro Gln Ala Trp Pro Leu Leu Leu
 180 185 190

Ala Ser Cys Leu Val Pro Gly Ala Leu Gln Leu Ala Ser Leu Pro Leu
 195 200 205

Leu Pro Glu Ser Pro Arg Tyr Leu Leu Ile Asp Cys Gly Asp Thr Glu
 210 215 220

Ala Cys Leu Ala Ala Leu Arg Arg Leu Arg Gly Ser Gly Asp Leu Ala
 225 230 235 240

Gly Glu Leu Glu Glu Leu Glu Glu Glu Arg Ala Ala Cys Gln Gly Cys
 245 250 255

Arg Ala Arg Arg Pro Trp Glu Leu Phe Gln His Arg Ala Leu Arg Arg
 260 265 270

Gln Val Thr Ser Leu Val Val Leu Gly Ser Ala Met Glu Leu Cys Gly
 275 280 285

Asn Asp Ser Val Tyr Ala Tyr Ala Ser Ser Val Phe Arg Lys Ala Gly
 290 295 300

Val Pro Glu Ala Lys Ile Gln Tyr Ala Ile Ile Gly Thr Gly Ser Cys
 305 310 315 320

Glu Leu Leu Thr Ala Val Val Ser Cys Val Val Ile Glu Arg Val Gly
 325 330 335

Arg Arg Val Leu Leu Ile Gly Gly Tyr Ser Leu Met Thr Cys Trp Gly
 340 345 350

Ser Ile Phe Thr Val Ala Leu Cys Leu Gln Ser Ser Phe Pro Trp Thr
 355 360 365

Leu Tyr Leu Ala Met Ala Cys Ile Phe Ala Phe Ile Leu Ser Phe Gly
 370 375 380

Ile Gly Pro Ala Gly Val Thr Gly Ile Leu Ala Thr Glu Leu Phe Asp
 385 390 395 400

Gln Met Ala Arg Pro Ala Ala Cys Met Val Cys Gly Ala Leu Met Trp
 405 410 415

Ile Met Leu Ile Leu Val Gly Leu Gly Phe Pro Phe Ile Met Glu Ala
 420 425 430

109

Leu Ser His Phe Leu Tyr Val Pro Phe Leu Gly Val Cys Val Cys Gly
 435 440 445

Ala Ile Tyr Thr Gly Leu Phe Leu Pro Glu Thr Lys Gly Lys Thr Phe
 450 455 460

Gln Glu Ile Ser Lys Glu Leu His Arg Leu Asn Phe Pro Arg Arg Ala
 465 470 475 480

Gln Gly Pro Thr Trp Arg Ser Leu Glu Val Ile Gln Ser Thr Glu Leu
 485 490 495

<210> 55
 <211> 2223
 <212> DNA
 <213> GLUT12

<220>
 <221> CDS
 <222> (106)..(1959)
 <223>

<400> 55
 acactcttct ttagcatgct attatgggga aagtgaccac tcctggggagc gggggtggtc 60

ggggcggttt ggtggcgggg aagcggctgt aacttctacg tgacc atg gta cct gtt 117
 Met Val Pro Val
 1

gaa aac acc gag ggc ccc agt ctg ctg aac cag aag ggg aca gcc gtg 165
 Glu Asn Thr Glu Gly Pro Ser Leu Leu Asn Gln Lys Gly Thr Ala Val
 5 10 15 20

gag acg gag ggc agc ggc agc cgg cat cct ccc tgg gcg aga ggc tgc 213
 Glu Thr Glu Gly Ser Gly Ser Arg His Pro Pro Trp Ala Arg Gly Cys
 25 30 35

ggc atg ttt acc ttc ctg tca tct gtc act gct gct gtc agt ggc ctc 261
 Gly Met Phe Thr Phe Leu Ser Ser Val Thr Ala Ala Val Ser Gly Leu
 40 45 50

ctg gtg ggt tat gaa ctt ggg atc atc tct ggg gct ctt ctt cag atc 309
 Leu Val Gly Tyr Glu Leu Gly Ile Ile Ser Gly Ala Leu Leu Gln Ile
 55 60 65

aaa acc tta tta gcc ctg agc tgc cat gag cag gaa atg gtt gtg agc 357
 Lys Thr Leu Leu Ala Leu Ser Cys His Glu Gln Glu Met Val Val Ser
 70 75 80

tcc ctc gtc att gga gcc ctc ctt gcc tca ctc acc gga ggg gtc ctg 405
 Ser Leu Val Ile Gly Ala Leu Leu Ala Ser Leu Thr Gly Gly Val Leu
 85 90 95 100

ata gac aga tat gga aga agg aca gca atc atc ttg tca tcc tgc ctg 453
 Ile Asp Arg Tyr Gly Arg Arg Thr Ala Ile Ile Leu Ser Ser Cys Leu
 105 110 115

ctt gga ctc gga agc tta gtc ttg atc ctc agt tta tcc tac acg gtt 501

Leu Gly Leu Gly Ser Leu Val Leu Ile Leu Ser Leu Ser Tyr Thr Val	
120 125 130	
ctt ata gtg gga cgc att gcc ata ggg gtc tcc atc tcc ctc tct tcc	549
Leu Ile Val Gly Arg Ile Ala Ile Gly Val Ser Ile Ser Leu Ser Ser	
135 140 145	
att gcc act tgt gtt tac atc gca gag att gct cct caa cac aga aga	597
Ile Ala Thr Cys Val Tyr Ile Ala Glu Ile Ala Pro Gln His Arg Arg	
150 155 160	
ggc ctt ctt gtg tca ctg aat gag ctg atg att gtc atc ggc att ctt	645
Gly Leu Leu Val Ser Leu Asn Glu Leu Met Ile Val Ile Gly Ile Leu	
165 170 175 180	
tct gcc tat att tca aat tac gca ttt gcc aat gtt ttc cat ggc tgg	693
Ser Ala Tyr Ile Ser Asn Tyr Ala Phe Ala Asn Val Phe His Gly Trp	
185 190 195	
aag tac atg ttt ggt ctt gtg att ccc ttg gga gtt ttg caa gca att	741
Lys Tyr Met Phe Gly Leu Val Ile Pro Leu Gly Val Leu Gln Ala Ile	
200 205 210	
gca atg tat ttt ctt cct cca agc cct cgg ttt ctg gtg atg aaa gga	789
Ala Met Tyr Phe Leu Pro Pro Ser Pro Arg Phe Leu Val Met Lys Gly	
215 220 225	
caa gag gga gct gct agc aag gtt ctt gga agg tta aga gca ctc tca	837
Gln Glu Gly Ala Ala Ser Lys Val Leu Gly Arg Leu Arg Ala Leu Ser	
230 235 240	
gat aca act gag gaa ctc act gtg atc aaa tcc tcc ctg aaa gat gaa	885
Asp Thr Thr Glu Glu Leu Thr Val Ile Lys Ser Ser Leu Lys Asp Glu	
245 250 255 260	
tat cag tac agt ttt tgg gat ctg ttt cgt tca aaa gac aac atg cgg	933
Tyr Gln Tyr Ser Phe Trp Asp Leu Phe Arg Ser Lys Asp Asn Met Arg	
265 270 275	
acc cga ata atg ata gga cta aca cta gta ttt ttt gta caa atc act	981
Thr Arg Ile Met Ile Gly Leu Thr Leu Val Phe Phe Val Gln Ile Thr	
280 285 290	
ggc caa cca aac ata ttg ttc tat gca tca act gtt ttg aag tca gtt	1029
Gly Gln Pro Asn Ile Leu Phe Tyr Ala Ser Thr Val Leu Lys Ser Val	
295 300 305	
gga ttt caa agc aat gag gca gct agc ctc gcc tcc act ggg gtt gga	1077
Gly Phe Gln Ser Asn Glu Ala Ala Ser Leu Ala Ser Thr Gly Val Gly	
310 315 320	
gtc gtc aag gtc att agc acc atc cct gcc act ctt ctt gta gac cat	1125
Val Val Lys Val Ile Ser Thr Ile Pro Ala Thr Leu Leu Val Asp His	
325 330 335 340	
gtt ggc agc aaa aca ttc ctc tgc att ggc tcc tct gtg atg gca gct	1173
Val Gly Ser Lys Thr Phe Leu Cys Ile Gly Ser Ser Val Met Ala Ala	
345 350 355	
tcg ttg gtg acc atg ggc atc gta aat ctc aac atc cac atg aac ttc	1221
Ser Leu Val Thr Met Gly Ile Val Asn Leu Asn Ile His Met Asn Phe	
360 365 370	

acc cat atc tgc aga agc cac aat tct atc aac cag tcc ttg gat gag	1269
Thr His Ile Cys Arg Ser His Asn Ser Ile Asn Gln Ser Leu Asp Glu	
375 380 385	
tct gtg att tat gga cca gga aac ctg tca acc aac aac aat act ctc	1317
Ser Val Ile Tyr Gly Pro Gly Asn Leu Ser Thr Asn Asn Asn Thr Leu	
390 395 400	
aga gac cac ttc aaa ggg att tct tcc cat agc aga agc tca ctc atg	1365
Arg Asp His Phe Lys Gly Ile Ser Ser His Ser Arg Ser Ser Leu Met	
405 410 415 420	
ccc ctg aga aat gat gtg gat aag aga ggg gag acg acc tca gca tcc	1413
Pro Leu Arg Asn Asp Val Asp Lys Arg Gly Glu Thr Thr Ser Ala Ser	
425 430 435	
ttg cta aat gct gga tta agc cac act gaa tac cag ata gtc aca gac	1461
Leu Leu Asn Ala Gly Leu Ser His Thr Glu Tyr Gln Ile Val Thr Asp	
440 445 450	
cct ggg gac gtc cca gct ttt ttg aaa tgg ctg tcc tta gcc agc ttg	1509
Pro Gly Asp Val Pro Ala Phe Leu Lys Trp Leu Ser Leu Ala Ser Leu	
455 460 465	
ctt gtt tat gtt gct gct ttt tca att ggt cta gga cca atg ccc tgg	1557
Leu Val Tyr Val Ala Ala Phe Ser Ile Gly Leu Gly Pro Met Pro Trp	
470 475 480	
ctg gtg ctc agc gag atc ttt cct ggt ggg atc aga gga cga gcc atg	1605
Leu Val Leu Ser Glu Ile Phe Pro Gly Gly Ile Arg Gly Arg Ala Met	
485 490 495 500	
gct tta act tct agc atg aac tgg ggc atc aat ctc ctc atc tcg ctg	1653
Ala Leu Thr Ser Ser Met Asn Trp Gly Ile Asn Leu Leu Ile Ser Leu	
505 510 515	
aca ttt ttg act gta act gat ctt att ggc ctg cca tgg gtg tgc ttt	1701
Thr Phe Leu Thr Val Thr Asp Leu Ile Gly Leu Pro Trp Val Cys Phe	
520 525 530	
ata tat aca atc atg agt cta gca tcc ctg ctt ttt gtt gtt atg ttt	1749
Ile Tyr Thr Ile Met Ser Leu Ala Ser Leu Leu Phe Val Val Met Phe	
535 540 545	
ata cct gag aca aag gga tgc tct ttg gaa caa ata tca atg gag cta	1797
Ile Pro Glu Thr Lys Gly Cys Ser Leu Glu Gln Ile Ser Met Glu Leu	
550 555 560	
gca aaa gtg aac tat gtg aaa aac aac att tgt ttt atg agt cat cac	1845
Ala Lys Val Asn Tyr Val Lys Asn Asn Ile Cys Phe Met Ser His His	
565 570 575 580	
caa gaa gaa tta gtg cca aaa cag cct caa aaa aga aaa ccc cag gag	1893
Gln Glu Glu Leu Val Pro Lys Gln Pro Gln Lys Arg Lys Pro Gln Glu	
585 590 595	
cag ctc ttg gag tgt aac aag ctg tgt ggt agg ggc caa tcc agg cag	1941
Gln Leu Leu Glu Cys Asn Lys Leu Cys Gly Arg Gly Gln Ser Arg Gln	
600 605 610	
ctt tct cca gag acc taa tggcctcaac accttctgaa cgtggatagt	1989
Leu Ser Pro Glu Thr	
615	

112

gccagaacac ttaggaggggt gtctttggac caatgcatag ttgcgactcc tgtgctctct 2049
 tttcagtgtc atggaactgg ttttgaagag acactctgaa atgataaaga cagcctttaa 2109
 tccccctcct ccccagaagg aacctcaaaa ggtagatgag gtacaaggtc ctaagtgatc 2169
 tcttttttctg agcaggatat caggcaaaaa aaaaaaaaaa aaaaaaaaaa aaaa 2223

<210> 56
 <211> 617
 <212> PRT
 <213> GLUT12

<400> 56

Met Val Pro Val Glu Asn Thr Glu Gly Pro Ser Leu Leu Asn Gln Lys
 1 5 10 15

Gly Thr Ala Val Glu Thr Glu Gly Ser Gly Ser Arg His Pro Pro Trp
 20 25 30

Ala Arg Gly Cys Gly Met Phe Thr Phe Leu Ser Ser Val Thr Ala Ala
 35 40 45

Val Ser Gly Leu Leu Val Gly Tyr Glu Leu Gly Ile Ile Ser Gly Ala
 50 55 60

Leu Leu Gln Ile Lys Thr Leu Leu Ala Leu Ser Cys His Glu Gln Glu
 65 70 75 80

Met Val Val Ser Ser Leu Val Ile Gly Ala Leu Leu Ala Ser Leu Thr
 85 90 95

Gly Gly Val Leu Ile Asp Arg Tyr Gly Arg Arg Thr Ala Ile Ile Leu
 100 105 110

Ser Ser Cys Leu Leu Gly Leu Gly Ser Leu Val Leu Ile Leu Ser Leu
 115 120 125

Ser Tyr Thr Val Leu Ile Val Gly Arg Ile Ala Ile Gly Val Ser Ile
 130 135 140

Ser Leu Ser Ser Ile Ala Thr Cys Val Tyr Ile Ala Glu Ile Ala Pro
 145 150 155 160

Gln His Arg Arg Gly Leu Leu Val Ser Leu Asn Glu Leu Met Ile Val
 165 170 175

Ile Gly Ile Leu Ser Ala Tyr Ile Ser Asn Tyr Ala Phe Ala Asn Val
 180 185 190

Phe His Gly Trp Lys Tyr Met Phe Gly Leu Val Ile Pro Leu Gly Val
 195 200 205

Leu Gln Ala Ile Ala Met Tyr Phe Leu Pro Pro Ser Pro Arg Phe Leu
 210 215 220

Val Met Lys Gly Gln Glu Gly Ala Ala Ser Lys Val Leu Gly Arg Leu
 225 230 235 240

Arg Ala Leu Ser Asp Thr Thr Glu Glu Leu Thr Val Ile Lys Ser Ser
 245 250 255

Leu Lys Asp Glu Tyr Gln Tyr Ser Phe Trp Asp Leu Phe Arg Ser Lys
 260 265 270

Asp Asn Met Arg Thr Arg Ile Met Ile Gly Leu Thr Leu Val Phe Phe
 275 280 285

Val Gln Ile Thr Gly Gln Pro Asn Ile Leu Phe Tyr Ala Ser Thr Val
 290 295 300

Leu Lys Ser Val Gly Phe Gln Ser Asn Glu Ala Ala Ser Leu Ala Ser
 305 310 315 320

Thr Gly Val Gly Val Val Lys Val Ile Ser Thr Ile Pro Ala Thr Leu
 325 330 335

Leu Val Asp His Val Gly Ser Lys Thr Phe Leu Cys Ile Gly Ser Ser
 340 345 350

Val Met Ala Ala Ser Leu Val Thr Met Gly Ile Val Asn Leu Asn Ile
 355 360 365

His Met Asn Phe Thr His Ile Cys Arg Ser His Asn Ser Ile Asn Gln
 370 375 380

Ser Leu Asp Glu Ser Val Ile Tyr Gly Pro Gly Asn Leu Ser Thr Asn
 385 390 395 400

Asn Asn Thr Leu Arg Asp His Phe Lys Gly Ile Ser Ser His Ser Arg
 405 410 415

Ser Ser Leu Met Pro Leu Arg Asn Asp Val Asp Lys Arg Gly Glu Thr
 420 425 430

Thr Ser Ala Ser Leu Leu Asn Ala Gly Leu Ser His Thr Glu Tyr Gln

<400> 57
ctgccggccc gcgagaccgc gtcccccccg gcagcccccg gccctgcc tatgtccgc 60
aaggcaagcg agaattgtgga gtacacgctg cggagcctga gcagcctg atg ggc gag 117
Met Gly Glu
1

115

cgg	cgc	agg	aag	cag	ccg	gag	ccg	gac	gcg	gcg	agc	gcg	gcc	ggg	gag	165
Arg	Arg	Arg	Lys	Gln	Pro	Glu	Pro	Asp	Ala	Ala	Ser	Ala	Ala	Gly	Glu	
5						10					15					
tgc	agc	ctc	ctg	gct	gcc	gcc	gaa	tcg	agc	acc	agc	ctg	cag	agc	gcg	213
Cys	Ser	Leu	Leu	Ala	Ala	Ala	Glu	Ser	Ser	Thr	Ser	Leu	Gln	Ser	Ala	
20				25					30						35	
ggc	gcg	ggc	ggc	ggc	ggc	gtc	ggg	gac	ctg	gag	cgc	gcg	gcg	cgg	cgg	261
Gly	Ala	Gly	Gly	Gly	Gly	Val	Gly	Asp	Leu	Glu	Arg	Ala	Ala	Arg	Arg	
				40				45						50		
cag	ttc	cag	cag	gac	gag	acc	ccc	gcc	ttc	gtg	tac	gtg	gtg	gcc	gtc	309
Gln	Phe	Gln	Gln	Asp	Glu	Thr	Pro	Ala	Phe	Val	Tyr	Val	Val	Ala	Val	
		55					60					65				
ttc	tcc	gcg	ctg	ggc	ggc	ttc	ctg	ttt	ggc	tat	gac	acc	ggg	gtg	gtg	357
Phe	Ser	Ala	Leu	Gly	Gly	Phe	Leu	Phe	Gly	Tyr	Asp	Thr	Gly	Val	Val	
	70					75					80					
tca	ggg	gcc	atg	ctg	ctg	ctc	aag	cgg	cag	ctc	agt	ctg	gac	gcg	ctg	405
Ser	Gly	Ala	Met	Leu	Leu	Leu	Lys	Arg	Gln	Leu	Ser	Leu	Asp	Ala	Leu	
85						90					95					
tgg	cag	gag	ctg	ctg	gtg	tcc	agc	acg	gtg	ggg	gcg	gct	gcc	gtc	tcg	453
Trp	Gln	Glu	Leu	Leu	Val	Ser	Ser	Thr	Val	Gly	Ala	Ala	Ala	Val	Ser	
100					105					110					115	
gcg	ctg	gcc	gga	ggc	gcc	ctc	aac	ggc	gtc	ttc	ggc	cgc	cgc	gct	gcc	501
Ala	Leu	Ala	Gly	Gly	Ala	Leu	Asn	Gly	Val	Phe	Gly	Arg	Arg	Ala	Ala	
			120					125						130		
atc	ctc	ctg	gcc	agt	gcc	ctc	ttc	acc	gcc	ggc	tcc	gcg	gtg	ctg	gct	549
Ile	Leu	Leu	Ala	Ser	Ala	Leu	Phe	Thr	Ala	Gly	Ser	Ala	Val	Leu	Ala	
		135					140						145			
gcg	gcc	aac	aac	aag	gag	aca	ctg	ctc	gcc	ggc	cgc	ctg	gtc	gtg	gga	597
Ala	Ala	Asn	Asn	Lys	Glu	Thr	Leu	Leu	Ala	Gly	Arg	Leu	Val	Val	Gly	
		150					155					160				
ctc	ggc	atc	ggc	att	gct	tct	atg	aca	gtg	cca	gtg	tac	att	gcg	gag	645
Leu	Gly	Ile	Gly	Ile	Ala	Ser	Met	Thr	Val	Pro	Val	Tyr	Ile	Ala	Glu	
	165					170					175					
gtc	tca	cca	ccc	aat	tta	aga	ggc	cga	tta	gtc	acc	att	aat	acc	ctc	693
Val	Ser	Pro	Pro	Asn	Leu	Arg	Gly	Arg	Leu	Val	Thr	Ile	Asn	Thr	Leu	
180					185					190					195	
ttc	atc	aca	gga	ggg	cag	ttc	ttt	gca	agt	gtt	gtt	gat	gga	gcc	ttc	741
Phe	Ile	Thr	Gly	Gly	Gln	Phe	Phe	Ala	Ser	Val	Val	Asp	Gly	Ala	Phe	
			200					205						210		
agt	tat	ctc	cag	aag	gat	gga	tgg	agg	tac	atg	ttg	gga	ctt	gca	rca	789
Ser	Tyr	Leu	Gln	Lys	Asp	Gly	Trp	Arg	Tyr	Met	Leu	Gly	Leu	Ala	Xaa	
		215					220					225				
gtt	ccg	gcg	gtt	ata	cag	ttt	ttt	ggc	ttt	ctc	ttt	ttg	cct	gaa	agc	837
Val	Pro	Ala	Val	Ile	Gln	Phe	Phe	Gly	Phe	Leu	Phe	Leu	Pro	Glu	Ser	
	230					235						240				
cct	cga	tgg	ctt	att	cag	aaa	gga	cag	act	cag	aag	gcc	cgt	aga	att	885
Pro	Arg	Trp	Leu	Ile	Gln	Lys	Gly	Gln	Thr	Gln	Lys	Ala	Arg	Arg	Ile	

245	250	255	
tta tct cag atg cgt ggt aac cag acc att gat gag gaa tat gat agc			933
Leu Ser Gln Met Arg Gly Asn Gln Thr Ile Asp Glu Glu Tyr Asp Ser			
260	265	270	275
atc aaa aac aac att gaa gag gag gaa aaa gag gtt ggc tca gct gga			981
Ile Lys Asn Asn Ile Glu Glu Glu Glu Lys Glu Val Gly Ser Ala Gly			
	280	285	290
cct gtg atc tgc aga atg ctg agt tat ccc cca act cgc cga gct tta			1029
Pro Val Ile Cys Arg Met Leu Ser Tyr Pro Pro Thr Arg Arg Ala Leu			
	295	300	305
att gtg ggt tgt ggc cta caa atg ttc cag cag ctc tca ggc att aac			1077
Ile Val Gly Cys Gly Leu Gln Met Phe Gln Gln Leu Ser Gly Ile Asn			
	310	315	320
acc atc atg tac tac agt gca acc att ctg cag atg tct ggt gtt gaa			1125
Thr Ile Met Tyr Tyr Ser Ala Thr Ile Leu Gln Met Ser Gly Val Glu			
	325	330	335
gat gat aga ctt gca ata tgg ctg gct tca gtt aca gcc ttc aca aat			1173
Asp Asp Arg Leu Ala Ile Trp Leu Ala Ser Val Thr Ala Phe Thr Asn			
340	345	350	355
ttc att ttc aca ctt gtg gga gtc tgg ctt gtt gag aag gtg ggc cgc			1221
Phe Ile Phe Thr Leu Val Gly Val Trp Leu Val Glu Lys Val Gly Arg			
	360	365	370
aga aag ctt acc ttt ggt agt tta gca ggt acc acc gta gca ctc att			1269
Arg Lys Leu Thr Phe Gly Ser Leu Ala Gly Thr Thr Val Ala Leu Ile			
	375	380	385
att ctt gcc ttg gga ttt gtg cta tca gcc caa gtt tcc cca cgc atc			1317
Ile Leu Ala Leu Gly Phe Val Leu Ser Ala Gln Val Ser Pro Arg Ile			
	390	395	400
act ttt aag cca ata gct ccg tca ggt cag aac gcc act tgc aca aga			1365
Thr Phe Lys Pro Ile Ala Pro Ser Gly Gln Asn Ala Thr Cys Thr Arg			
	405	410	415
tac agt tac tgt aat gaa tgt atg ttg gat cca gac tgc ggt ttc tgc			1413
Tyr Ser Tyr Cys Asn Glu Cys Met Leu Asp Pro Asp Cys Gly Phe Cys			
	420	425	430
twc aag atg aac aaa tca act gtc att gac tcc tcc tgt gtt cca gtt			1461
Xaa Lys Met Asn Lys Ser Thr Val Ile Asp Ser Ser Cys Val Pro Val			
	440	445	450
aat aaa gca tct aca aat gag gca gcc tgg ggc agg tgt gaa aat gaa			1509
Asn Lys Ala Ser Thr Asn Glu Ala Ala Trp Gly Arg Cys Glu Asn Glu			
	455	460	465
acc aag ttc aaa aca gaa gat ata ttt tgg gct tac aat ttc tgc cct			1557
Thr Lys Phe Lys Thr Glu Asp Ile Phe Trp Ala Tyr Asn Phe Cys Pro			
	470	475	480
act cca tac tcc tgg act gca ctt ctg ggc ctt att tta tat ctt gtc			1605
Thr Pro Tyr Ser Trp Thr Ala Leu Leu Gly Leu Ile Leu Tyr Leu Val			
	485	490	495
ttc ttt gca cct gga atg gga cca atg cct tgg act gtg aat tct gaa			1653

118

```

gcccaaatca ctacaccttc ctcatgggcc ttggagtcct actattttgt gccttcattt 2938
gtgttacata catagctgca agcaaaccat ttttcccctt tcttttattc aaacaataat 2998
ttttgaaaca aaaaagagga aggaaatcag tggcagaaat aatcctgctg ttattggtgt 3058
ttgtttaata aaaataatgg gacttttttc ttaacttttt attagctcct cctaaggga 3118
atgtcacata ttattattta attgtacttg tcttttttta ctttaagagc ataaactcgt 3178
ttttattttg cacacttttc tcattttcct gagaatttac cagaaaaaaa aagatacata 3238
gatttgcttc tgtgtttttc.tta 3261

```

```

<210> 58
<211> 629
<212> PRT
<213> GLUT13 (HMIT)

<220>
<221> misc_feature
<222> (227)..(227)
<223> The 'Xaa' at location 227 stands for Ala, or Thr.

```

```

<220>
<221> misc_feature
<222> (436)..(436)
<223> The 'Xaa' at location 436 stands for Tyr, or Phe.

```

```

<400> 58

```

```

Met Gly Glu Arg Arg Arg Lys Gln Pro Glu Pro Asp Ala Ala Ser Ala
1          5          10          15

```

```

Ala Gly Glu Cys Ser Leu Leu Ala Ala Ala Glu Ser Ser Thr Ser Leu
20          25          30

```

```

Gln Ser Ala Gly Ala Gly Gly Gly Val Gly Asp Leu Glu Arg Ala
35          40          45

```

```

Ala Arg Arg Gln Phe Gln Gln Asp Glu Thr Pro Ala Phe Val Tyr Val
50          55          60

```

```

Val Ala Val Phe Ser Ala Leu Gly Gly Phe Leu Phe Gly Tyr Asp Thr
65          70          75          80

```

```

Gly Val Val Ser Gly Ala Met Leu Leu Leu Lys Arg Gln Leu Ser Leu
85          90          95

```

```

Asp Ala Leu Trp Gln Glu Leu Leu Val Ser Ser Thr Val Gly Ala Ala
100         105         110

```

```

Ala Val Ser Ala Leu Ala Gly Gly Ala Leu Asn Gly Val Phe Gly Arg
115         120         125

```

119

Arg Ala Ala Ile Leu Leu Ala Ser Ala Leu Phe Thr Ala Gly Ser Ala
 130 135 140

Val Leu Ala Ala Ala Asn Asn Lys Glu Thr Leu Leu Ala Gly Arg Leu
 145 150 155 160

Val Val Gly Leu Gly Ile Gly Ile Ala Ser Met Thr Val Pro Val Tyr
 165 170 175

Ile Ala Glu Val Ser Pro Pro Asn Leu Arg Gly Arg Leu Val Thr Ile
 180 185 190

Asn Thr Leu Phe Ile Thr Gly Gly Gln Phe Phe Ala Ser Val Val Asp
 195 200 205

Gly Ala Phe Ser Tyr Leu Gln Lys Asp Gly Trp Arg Tyr Met Leu Gly
 210 215 220

Leu Ala Xaa Val Pro Ala Val Ile Gln Phe Phe Gly Phe Leu Phe Leu
 225 230 235 240

Pro Glu Ser Pro Arg Trp Leu Ile Gln Lys Gly Gln Thr Gln Lys Ala
 245 250 255

Arg Arg Ile Leu Ser Gln Met Arg Gly Asn Gln Thr Ile Asp Glu Glu
 260 265 270

Tyr Asp Ser Ile Lys Asn Asn Ile Glu Glu Glu Glu Lys Glu Val Gly
 275 280 285

Ser Ala Gly Pro Val Ile Cys Arg Met Leu Ser Tyr Pro Pro Thr Arg
 290 295 300

Arg Ala Leu Ile Val Gly Cys Gly Leu Gln Met Phe Gln Gln Leu Ser
 305 310 315 320

Gly Ile Asn Thr Ile Met Tyr Tyr Ser Ala Thr Ile Leu Gln Met Ser
 325 330 335

Gly Val Glu Asp Asp Arg Leu Ala Ile Trp Leu Ala Ser Val Thr Ala
 340 345 350

Phe Thr Asn Phe Ile Phe Thr Leu Val Gly Val Trp Leu Val Glu Lys
 355 360 365

Val Gly Arg Arg Lys Leu Thr Phe Gly Ser Leu Ala Gly Thr Thr Val
 370 375 380

120

Ala Leu Ile Ile Leu Ala Leu Gly Phe Val Leu Ser Ala Gln Val Ser
385 390 395 400

Pro Arg Ile Thr Phe Lys Pro Ile Ala Pro Ser Gly Gln Asn Ala Thr
405 410 415

Cys Thr Arg Tyr Ser Tyr Cys Asn Glu Cys Met Leu Asp Pro Asp Cys
420 425 430

Gly Phe Cys Xaa Lys Met Asn Lys Ser Thr Val Ile Asp Ser Ser Cys
435 440 445

Val Pro Val Asn Lys Ala Ser Thr Asn Glu Ala Ala Trp Gly Arg Cys
450 455 460

Glu Asn Glu Thr Lys Phe Lys Thr Glu Asp Ile Phe Trp Ala Tyr Asn
465 470 475 480

Phe Cys Pro Thr Pro Tyr Ser Trp Thr Ala Leu Leu Gly Leu Ile Leu
485 490 495

Tyr Leu Val Phe Phe Ala Pro Gly Met Gly Pro Met Pro Trp Thr Val
500 505 510

Asn Ser Glu Ile Tyr Pro Leu Trp Ala Arg Ser Thr Gly Asn Ala Cys
515 520 525

Ser Ser Gly Ile Asn Trp Ile Phe Asn Val Leu Val Ser Leu Thr Phe
530 535 540

Leu His Thr Ala Glu Tyr Leu Thr Tyr Tyr Gly Ala Phe Phe Leu Tyr
545 550 555 560

Ala Gly Phe Ala Ala Val Gly Leu Leu Phe Ile Tyr Gly Cys Leu Pro
565 570 575

Glu Thr Lys Gly Lys Lys Leu Glu Glu Ile Glu Ser Leu Phe Asp Asn
580 585 590

Arg Leu Cys Thr Cys Gly Thr Ser Asp Ser Asp Glu Gly Arg Tyr Ile
595 600 605

Glu Tyr Ile Arg Val Lys Gly Ser Asn Tyr His Leu Ser Asp Asn Asp
610 615 620

Ala Ser Asp Val Glu

625

<210> 59
 <211> 2118
 <212> DNA
 <213> GLUT14

<220>
 <221> CDS
 <222> (110)..(1603)
 <223>

<400> 59
 agcggggctg agcgaagccg cggggcccaa ccgcagtcgc ggggtctggg aggaagcagt 60
 accttgaaga gaaattggag agggagtcaa ttcctaggat agcagagag atg gac aac 118
 Met Asp Asn
 1
 aga cag aat gtc acc cca gct ctg atc ttt gcc atc aca gtt gct aca 166
 Arg Gln Asn Val Thr Pro Ala Leu Ile Phe Ala Ile Thr Val Ala Thr
 5 10 15
 atc gcc tct ttc cag ttt gcc tac aac act ggg gtc atc aat gct cct 214
 Ile Gly Ser Phe Gln Phe Gly Tyr Asn Thr Gly Val Ile Asn Ala Pro
 20 25 30 35
 gag acg atc ata aag gaa ttt atc aat aaa act ttg acg gac aag gca 262
 Glu Thr Ile Ile Lys Glu Phe Ile Asn Lys Thr Leu Thr Asp Lys Ala
 40 45 50
 aat gcc cct ccc tct gag gtg ctg ctc acg aat ctc tgg tcc ttg tct 310
 Asn Ala Pro Pro Ser Glu Val Leu Leu Thr Asn Leu Trp Ser Leu Ser
 55 60 65
 gtg gcc ata ttt tcc gtc ggg ggt atg atc gcc tcc ttt tcc gtc gga 358
 Val Ala Ile Phe Ser Val Gly Gly Met Ile Gly Ser Phe Ser Val Gly
 70 75 80
 ctc ttt gtt aac cgc ttt ggc agg cgc aat tca atg ctg att gtc aac 406
 Leu Phe Val Asn Arg Phe Gly Arg Arg Asn Ser Met Leu Ile Val Asn
 85 90 95
 ctg ttg gct gcc act ggt ggc tgc ctt atg gga ctg tgt aaa ata gct 454
 Leu Leu Ala Ala Thr Gly Gly Cys Leu Met Gly Leu Cys Lys Ile Ala
 100 105 110 115
 gag tca gtt gaa atg ctg atc ctg ggc cgc ttg gtt att ggc ctc ttc 502
 Glu Ser Val Glu Met Leu Ile Leu Gly Arg Leu Val Ile Gly Leu Phe
 120 125 130
 tgc gga ctc tgc aca ggt ttt gtg ccc atg tac att gga gag atc tcg 550
 Cys Gly Leu Cys Thr Gly Phe Val Pro Met Tyr Ile Gly Glu Ile Ser
 135 140 145
 cct act gcc ctg agg ggt gcc ttt ggc act ctc aac cag ctg ggc ata 598
 Pro Thr Ala Leu Arg Gly Ala Phe Gly Thr Leu Asn Gln Leu Gly Ile
 150 155 160
 gtt att gga att ctg gtg gcc cag atc ttt ggt ctg gaa ctc atc ctt 646
 Val Ile Gly Ile Leu Val Ala Gln Ile Phe Gly Leu Glu Leu Ile Leu
 165 170 175

ggg tct gaa gag cta tgg ccg gtg cta tta ggc ttt acc atc ctt cca Gly Ser Glu Glu Leu Trp Pro Val Leu Leu Gly Phe Thr Ile Leu Pro 180 185 190 195	694
gct atc ctg caa agt gca gcc ctt cca tgt tgc cct gaa agt ccc aga Ala Ile Leu Gln Ser Ala Ala Leu Pro Cys Cys Pro Glu Ser Pro Arg 200 205 210	742
ttt ttg ctc att aac aga aaa aaa gag gag aat gct acg cgg atc ctc Phe Leu Leu Ile Asn Arg Lys Lys Glu Glu Asn Ala Thr Arg Ile Leu 215 220 225	790
cag cgg ttg tgg ggc acc cag gat gta tcc caa gac atc cag gag atg Gln Arg Leu Trp Gly Thr Gln Asp Val Ser Gln Asp Ile Gln Glu Met 230 235 240	838
aaa gat gag agt gca agg atg tca caa gaa aag caa gtc acc gtg ctg Lys Asp Glu Ser Ala Arg Met Ser Gln Glu Lys Gln Val Thr Val Leu 245 250 255	886
gag ctc ttt aga gtg tcc agc tac cga cag ccc atc atc att tcc att Glu Leu Phe Arg Val Ser Ser Tyr Arg Gln Pro Ile Ile Ile Ser Ile 260 265 270 275	934
gtg ctc cag ctc tct cag cag ctc tct ggg atc aat gct gtg ttc tat Val Leu Gln Leu Ser Gln Gln Leu Ser Gly Ile Asn Ala Val Phe Tyr 280 285 290	982
tac tca aca gga atc ttc aag gat gca ggt gtt caa cag ccc atc tat Tyr Ser Thr Gly Ile Phe Lys Asp Ala Gly Val Gln Gln Pro Ile Tyr 295 300 305	1030
gcc acc atc agc gcg ggt gtg gtt aat act atc ttc act tta ctt tct Ala Thr Ile Ser Ala Gly Val Val Asn Thr Ile Phe Thr Leu Leu Ser 310 315 320	1078
cta ttt ctg gtg gaa agg gca gga aga agg act ctg cat atg ata ggc Leu Phe Leu Val Glu Arg Ala Gly Arg Arg Thr Leu His Met Ile Gly 325 330 335	1126
ctt gga ggg atg gct ttt tgt tcc acg ctc atg act gtt tct ttg tta Leu Gly Gly Met Ala Phe Cys Ser Thr Leu Met Thr Val Ser Leu Leu 340 345 350 355	1174
tta aag aat cac tat aat ggg atg agc ttt gtc tgt att ggg gct atc Leu Lys Asn His Tyr Asn Gly Met Ser Phe Val Cys Ile Gly Ala Ile 360 365 370	1222
ttg gtc ttt gtg gcc tgt ttt gaa att gga cca ggc ccc att ccc tgg Leu Val Phe Val Ala Cys Phe Glu Ile Gly Pro Gly Pro Ile Pro Trp 375 380 385	1270
ttt att gtg gcc gaa ctc ttc agc cag ggc ccc cgc cca gct gcg atg Phe Ile Val Ala Glu Leu Phe Ser Gln Gly Pro Arg Pro Ala Ala Met 390 395 400	1318
gca gtg gcc ggc tgc tcc aac tgg acc tcc aac ttc cta gtc gga ttg Ala Val Ala Gly Cys Ser Asn Trp Thr Ser Asn Phe Leu Val Gly Leu 405 410 415	1366
ctc ttc ccc tct gct gct tac tat tta gga gcc tac gtt ttt att atc Leu Phe Pro Ser Ala Ala Tyr Tyr Leu Gly Ala Tyr Val Phe Ile Ile	1414

123

420 425 430 435
 ttc acc ggc ttc ctc att acc ttc ttg gcc ttt acc ttc ttc aaa gtc 1462
 Phe Thr Gly Phe Leu Ile Thr Phe Leu Ala Phe Thr Phe Phe Lys Val
 440 445 450
 cct gag acc cgt ggc agg act ttt gag gat atc aca cgg gcc ttt gaa 1510
 Pro Glu Thr Arg Gly Arg Thr Phe Glu Asp Ile Thr Arg Ala Phe Glu
 455 460 465
 ggg cag gca cac ggt gca gat aga tct ggg aag gac ggc gtc atg ggg 1558
 Gly Gln Ala His Gly Ala Asp Arg Ser Gly Lys Asp Gly Val Met Gly
 470 475 480
 atg aac agc atc gag cct gct aag gag acc acc acc aat gtc taa 1603
 Met Asn Ser Ile Glu Pro Ala Lys Glu Thr Thr Thr Asn Val
 485 490 495
 gtcatgcctc cttccacctc cctcccggca tgggaaagcc acctctccct caacaaggga 1663
 gagactttat caggatgaac ccaggactgc ttctgaatgc tgctacttga tttctttctc 1723
 atcccacgca ctccatgagc accccaaggc tgcagtttgt tggatcttca atggcttttt 1783
 aaattttatt tcctggacat cctcttctgc ttaggagaga ccgagtgaac ctaccttcat 1843
 ttcaggaggg attggccgct tggcacatga caactttgcc agcttttcct cccttggggtt 1903
 ctgatattgc cgcactagag gatataggag aggaaaagta aggtgcagtt gccccaacct 1963
 cagacttacc aggaagcaga tacatatgag tgtggaagcc ggaggggtgtt tatgtaagag 2023
 cacccttctc acttccatac agctctacgc ggcaaattaa cttgagtttt atttatctta 2083
 tcctctgggtt taattacata aatattttatt tttta 2118

 <210> 60
 <211> 497
 <212> PRT
 <213> GLUT14

 <400> 60

 Met Asp Asn Arg Gln Asn Val Thr Pro Ala Leu Ile Phe Ala Ile Thr
 1 5 10 15

 Val Ala Thr Ile Gly Ser Phe Gln Phe Gly Tyr Asn Thr Gly Val Ile
 20 25 30

 Asn Ala Pro Glu Thr Ile Ile Lys Glu Phe Ile Asn Lys Thr Leu Thr
 35 40 45

 Asp Lys Ala Asn Ala Pro Pro Ser Glu Val Leu Leu Thr Asn Leu Trp
 50 55 60

 Ser Leu Ser Val Ala Ile Phe Ser Val Gly Gly Met Ile Gly Ser Phe
 65 70 75 80

124

Ser Val Gly Leu Phe Val Asn Arg Phe Gly Arg Arg Asn Ser Met Leu
 85 90 95

Ile Val Asn Leu Leu Ala Ala Thr Gly Gly Cys Leu Met Gly Leu Cys
 100 105 110

Lys Ile Ala Glu Ser Val Glu Met Leu Ile Leu Gly Arg Leu Val Ile
 115 120 125

Gly Leu Phe Cys Gly Leu Cys Thr Gly Phe Val Pro Met Tyr Ile Gly
 130 135 140

Glu Ile Ser Pro Thr Ala Leu Arg Gly Ala Phe Gly Thr Leu Asn Gln
 145 150 155 160

Leu Gly Ile Val Ile Gly Ile Leu Val Ala Gln Ile Phe Gly Leu Glu
 165 170 175

Leu Ile Leu Gly Ser Glu Glu Leu Trp Pro Val Leu Leu Gly Phe Thr
 180 185 190

Ile Leu Pro Ala Ile Leu Gln Ser Ala Ala Leu Pro Cys Cys Pro Glu
 195 200 205

Ser Pro Arg Phe Leu Leu Ile Asn Arg Lys Lys Glu Glu Asn Ala Thr
 210 215 220

Arg Ile Leu Gln Arg Leu Trp Gly Thr Gln Asp Val Ser Gln Asp Ile
 225 230 235 240

Gln Glu Met Lys Asp Glu Ser Ala Arg Met Ser Gln Glu Lys Gln Val
 245 250 255

Thr Val Leu Glu Leu Phe Arg Val Ser Ser Tyr Arg Gln Pro Ile Ile
 260 265 270

Ile Ser Ile Val Leu Gln Leu Ser Gln Gln Leu Ser Gly Ile Asn Ala
 275 280 285

Val Phe Tyr Tyr Ser Thr Gly Ile Phe Lys Asp Ala Gly Val Gln Gln
 290 295 300

Pro Ile Tyr Ala Thr Ile Ser Ala Gly Val Val Asn Thr Ile Phe Thr
 305 310 315 320

Leu Leu Ser Leu Phe Leu Val Glu Arg Ala Gly Arg Arg Thr Leu His
 325 330 335

125

Met Ile Gly Leu Gly Gly Met Ala Phe Cys Ser Thr Leu Met Thr Val
 340 345 350

Ser Leu Leu Leu Lys Asn His Tyr Asn Gly Met Ser Phe Val Cys Ile
 355 360 365

Gly Ala Ile Leu Val Phe Val Ala Cys Phe Glu Ile Gly Pro Gly Pro
 370 375 380

Ile Pro Trp Phe Ile Val Ala Glu Leu Phe Ser Gln Gly Pro Arg Pro
 385 390 395 400

Ala Ala Met Ala Val Ala Gly Cys Ser Asn Trp Thr Ser Asn Phe Leu
 405 410 415

Val Gly Leu Leu Phe Pro Ser Ala Ala Tyr Tyr Leu Gly Ala Tyr Val
 420 425 430

Phe Ile Ile Phe Thr Gly Phe Leu Ile Thr Phe Leu Ala Phe Thr Phe
 435 440 445

Phe Lys Val Pro Glu Thr Arg Gly Arg Thr Phe Glu Asp Ile Thr Arg
 450 455 460

Ala Phe Glu Gly Gln Ala His Gly Ala Asp Arg Ser Gly Lys Asp Gly
 465 470 475 480

Val Met Gly Met Asn Ser Ile Glu Pro Ala Lys Glu Thr Thr Thr Asn
 485 490 495

Val

<210> 61

<211> 6126

<212> DNA

<213> Cystic Fibrosis Transmembrane Conductor Regulator (CFTR) delta F508 mutation

<220>

<221> CDS

<222> (133)..(4572)

<223>

<400> 61

aattggaagc aaatgacatc acagcaggtc agagaaaaag ggttgagcgg caggcaccca 60

gagtagtagg tctttggcat taggagcttg agcccagacg gccctagcag ggacccacgc 120

gcccagagaga cc atg cag agg tcg cct ctg gaa aag gcc agc gtt gtc tcc 171

Met Gln Arg Ser Pro Leu Glu Lys Ala Ser Val Val Ser																
				1			5				10					
aaa	ctt	ttt	ttc	agc	tgg	acc	aga	cca	att	ttg	agg	aaa	gga	tac	aga	219
Lys	Leu	Phe	Phe	Ser	Trp	Thr	Arg	Pro	Ile	Leu	Arg	Lys	Gly	Tyr	Arg	
		15				20					25					
cag	cgc	ctg	gaa	ttg	tca	gac	ata	tac	caa	atc	cct	tct	gtt	gat	tct	267
Gln	Arg	Leu	Glu	Leu	Ser	Asp	Ile	Tyr	Gln	Ile	Pro	Ser	Val	Asp	Ser	
30					35					40					45	
gct	gac	aat	cta	tct	gaa	aaa	ttg	gaa	aga	gaa	tgg	gat	aga	gag	ctg	315
Ala	Asp	Asn	Leu	Ser	Glu	Lys	Leu	Glu	Arg	Glu	Trp	Asp	Arg	Glu	Leu	
				50					55					60		
gct	tca	aag	aaa	aat	cct	aaa	ctc	att	aat	gcc	ctt	cgg	cga	tgt	ttt	363
Ala	Ser	Lys	Lys	Asn	Pro	Lys	Leu	Ile	Asn	Ala	Leu	Arg	Arg	Cys	Phe	
			65					70					75			
ttc	tgg	aga	ttt	atg	ttc	tat	gga	atc	ttt	tta	tat	tta	ggg	gaa	gtc	411
Phe	Trp	Arg	Phe	Met	Phe	Tyr	Gly	Ile	Phe	Leu	Tyr	Leu	Gly	Glu	Val	
		80					85					90				
acc	aaa	gca	gta	cag	cct	ctc	tta	ctg	gga	aga	atc	ata	gct	tcc	tat	459
Thr	Lys	Ala	Val	Gln	Pro	Leu	Leu	Leu	Gly	Arg	Ile	Ile	Ala	Ser	Tyr	
		95				100					105					
gac	ccg	gat	aac	aag	gag	gaa	cgc	tct	atc	gcg	att	tat	cta	ggc	ata	507
Asp	Pro	Asp	Asn	Lys	Glu	Glu	Arg	Ser	Ile	Ala	Ile	Tyr	Leu	Gly	Ile	
110					115					120					125	
ggc	tta	tgc	ctt	ctc	ttt	att	gtg	agg	aca	ctg	ctc	cta	cac	cca	gcc	555
Gly	Leu	Cys	Leu	Leu	Phe	Ile	Val	Arg	Thr	Leu	Leu	Leu	His	Pro	Ala	
				130					135					140		
att	ttt	ggc	ctt	cat	cac	att	gga	atg	cag	atg	aga	ata	gct	atg	ttt	603
Ile	Phe	Gly	Leu	His	His	Ile	Gly	Met	Gln	Met	Arg	Ile	Ala	Met	Phe	
			145					150					155			
agt	ttg	att	tat	aag	aag	act	tta	aag	ctg	tca	agc	cgt	gtt	cta	gat	651
Ser	Leu	Ile	Tyr	Lys	Lys	Thr	Leu	Lys	Leu	Ser	Ser	Arg	Val	Leu	Asp	
		160					165					170				
aaa	ata	agt	att	gga	caa	ctt	gtt	agt	ctc	ctt	tcc	aac	aac	ctg	aac	699
Lys	Ile	Ser	Ile	Gly	Gln	Leu	Val	Ser	Leu	Leu	Ser	Asn	Asn	Leu	Asn	
		175				180					185					
aaa	ttt	gat	gaa	gga	ctt	gca	ttg	gca	cat	ttc	gtg	tgg	atc	gct	cct	747
Lys	Phe	Asp	Glu	Gly	Leu	Ala	Leu	Ala	His	Phe	Val	Trp	Ile	Ala	Pro	
190					195					200					205	
ttg	caa	gtg	gca	ctc	ctc	atg	ggg	cta	atc	tgg	gag	ttg	tta	cag	gcg	795
Leu	Gln	Val	Ala	Leu	Leu	Met	Gly	Leu	Ile	Trp	Glu	Leu	Leu	Gln	Ala	
				210					215					220		
tct	gcc	ttc	tgt	gga	ctt	ggt	ttc	ctg	ata	gtc	ctt	gcc	ctt	ttt	cag	843
Ser	Ala	Phe	Cys	Gly	Leu	Gly	Phe	Leu	Ile	Val	Leu	Ala	Leu	Phe	Gln	
			225					230					235			
gct	ggg	cta	ggg	aga	atg	atg	atg	aag	tac	aga	gat	cag	aga	gct	ggg	891
Ala	Gly	Leu	Gly	Arg	Met	Met	Met	Lys	Tyr	Arg	Asp	Gln	Arg	Ala	Gly	
		240					245					250				

aag atc agt gaa aga ctt gtg att acc tca gaa atg att gaa aat atc Lys Ile Ser Glu Arg Leu Val Ile Thr Ser Glu Met Ile Glu Asn Ile 255 260 265	939
caa tct gtt aag gca tac tgc tgg gaa gaa gca atg gaa aaa atg att Gln Ser Val Lys Ala Tyr Cys Trp Glu Glu Ala Met Glu Lys Met Ile 270 275 280 285	987
gaa aac tta aga caa aca gaa ctg aaa ctg act cgg aag gca gcc tat Glu Asn Leu Arg Gln Thr Glu Leu Lys Leu Thr Arg Lys Ala Ala Tyr 290 295 300	1035
gtg aga tac ttc aat agc tca gcc ttc ttc ttc tca ggg ttc ttt gtg Val Arg Tyr Phe Asn Ser Ser Ala Phe Phe Phe Ser Gly Phe Phe Val 305 310 315	1083
gtg ttt tta tct gtg ctt ccc tat gca cta atc aaa gga atc atc ctc Val Phe Leu Ser Val Leu Pro Tyr Ala Leu Ile Lys Gly Ile Ile Leu 320 325 330	1131
cgg aaa ata ttc acc acc atc tca ttc tgc att gtt ctg cgc atg gcg Arg Lys Ile Phe Thr Thr Ile Ser Phe Cys Ile Val Leu Arg Met Ala 335 340 345	1179
gtc act cgg caa ttt ccc tgg gct gta caa aca tgg tat gac tct ctt Val Thr Arg Gln Phe Pro Trp Ala Val Gln Thr Trp Tyr Asp Ser Leu 350 355 360 365	1227
gga gca ata aac aaa ata cag gat ttc tta caa aag caa gaa tat aag Gly Ala Ile Asn Lys Ile Gln Asp Phe Leu Gln Lys Gln Glu Tyr Lys 370 375 380	1275
aca ttg gaa tat aac tta acg act aca gaa gta gtg atg gag aat gta Thr Leu Glu Tyr Asn Leu Thr Thr Thr Glu Val Val Met Glu Asn Val 385 390 395	1323
aca gcc ttc tgg gag gag gga ttt ggg gaa tta ttt gag aaa gca aaa Thr Ala Phe Trp Glu Glu Gly Phe Gly Glu Leu Phe Glu Lys Ala Lys 400 405 410	1371
caa aac aat aac aat aga aaa act tct aat ggt gat gac agc ctc ttc Gln Asn Asn Asn Asn Arg Lys Thr Ser Asn Gly Asp Asp Ser Leu Phe 415 420 425	1419
ttc agt aat ttc tca ctt ctt ggt act cct gtc ctg aaa gat att aat Phe Ser Asn Phe Ser Leu Leu Gly Thr Pro Val Leu Lys Asp Ile Asn 430 435 440 445	1467
ttc aag ata gaa aga gga cag ttg ttg gcg gtt gct gga tcc act gga Phe Lys Ile Glu Arg Gly Gln Leu Leu Ala Val Ala Gly Ser Thr Gly 450 455 460	1515
gca ggc aag act tca ctt cta atg atg att atg gga gaa ctg gag cct Ala Gly Lys Thr Ser Leu Leu Met Met Ile Met Gly Glu Leu Glu Pro 465 470 475	1563
tca gag ggt aaa att aag cac agt gga aga att tca ttc tgt tct cag Ser Glu Gly Lys Ile Lys His Ser Gly Arg Ile Ser Phe Cys Ser Gln 480 485 490	1611
ttt tcc tgg att atg cct ggc acc att aaa gaa aat atc atc ggt gtt Phe Ser Trp Ile Met Pro Gly Thr Ile Lys Glu Asn Ile Ile Gly Val 495 500 505	1659

tcc tat gat gaa tat aga tac aga agc gtc atc aaa gca tgc caa cta Ser Tyr Asp Glu Tyr Arg Tyr Arg Ser Val Ile Lys Ala Cys Gln Leu 510 515 520 525	1707
gaa gag gac atc tcc aag ttt gca gag aaa gac aat ata gtt ctt gga Glu Glu Asp Ile Ser Lys Phe Ala Glu Lys Asp Asn Ile Val Leu Gly 530 535 540	1755
gaa ggt gga atc aca ctg agt gga ggt caa cga gca aga att tct tta Glu Gly Gly Ile Thr Leu Ser Gly Gly Gln Arg Ala Arg Ile Ser Leu 545 550 555	1803
gca aga gca gta tac aaa gat gct gat ttg tat tta tta gac tct cct Ala Arg Ala Val Tyr Lys Asp Ala Asp Leu Tyr Leu Leu Asp Ser Pro 560 565 570	1851
ttt gga tac cta gat gtt tta aca gaa aaa gaa ata ttt gaa agc tgt Phe Gly Tyr Leu Asp Val Leu Thr Glu Lys Glu Ile Phe Glu Ser Cys 575 580 585	1899
gtc tgt aaa ctg atg gct aac aaa act agg att ttg gtc act tct aaa Val Cys Lys Leu Met Ala Asn Lys Thr Arg Ile Leu Val Thr Ser Lys 590 595 600 605	1947
atg gaa cat tta aag aaa gct gac aaa ata tta att ttg aat gaa ggt Met Glu His Leu Lys Lys Ala Asp Lys Ile Leu Ile Leu Asn Glu Gly 610 615 620	1995
agc agc tat ttt tat ggg aca ttt tca gaa ctc caa aat cta cag cca Ser Ser Tyr Phe Tyr Gly Thr Phe Ser Glu Leu Gln Asn Leu Gln Pro 625 630 635	2043
gac ttt agc tca aaa ctc atg gga tgt gat tct ttc gac caa ttt agt Asp Phe Ser Ser Lys Leu Met Gly Cys Asp Ser Phe Asp Gln Phe Ser 640 645 650	2091
gca gaa aga aga aat tca atc cta act gag acc tta cac cgt ttc tca Ala Glu Arg Arg Asn Ser Ile Leu Thr Glu Thr Leu His Arg Phe Ser 655 660 665	2139
tta gaa gga gat gct cct gtc tcc tgg aca gaa aca aaa aaa caa tct Leu Glu Gly Asp Ala Pro Val Ser Trp Thr Glu Thr Lys Lys Gln Ser 670 675 680 685	2187
ttt aaa cag act gga gag ttt ggg gaa aaa agg aag aat tct att ctc Phe Lys Gln Thr Gly Glu Phe Gly Glu Lys Arg Lys Asn Ser Ile Leu 690 695 700	2235
aat cca atc aac tct ata cga aaa ttt tcc att gtg caa aag act ccc Asn Pro Ile Asn Ser Ile Arg Lys Phe Ser Ile Val Gln Lys Thr Pro 705 710 715	2283
tta caa atg aat ggc atc gaa gag gat tct gat gag cct tta gag aga Leu Gln Met Asn Gly Ile Glu Glu Asp Ser Asp Glu Pro Leu Glu Arg 720 725 730	2331
agg ctg tcc tta gta cca gat tct gag cag gga gag gcg ata ctg cct Arg Leu Ser Leu Val Pro Asp Ser Glu Gln Gly Glu Ala Ile Leu Pro 735 740 745	2379
cgc atc agc gtg atc agc act ggc ccc acg ctt cag gca cga agg agg Arg Ile Ser Val Ile Ser Thr Gly Pro Thr Leu Gln Ala Arg Arg Arg	2427

750	755	760	765	
cag tct gtc ctg aac	ctg atg aca cac tca gtt aac	caa ggt cag aac	2475	
Gln Ser Val Leu	Asn Leu Met Thr His	Ser Val Asn Gln Gly	Gln Asn	
	770	775	780	
att cac cga aag aca	aca gca tcc aca cga aaa gtg tca	ctg gcc cct	2523	
Ile His Arg Lys	Thr Thr Ala Ser Thr Arg Lys Val	Ser Leu Ala Pro		
	785	790	795	
cag gca aac ttg act	gaa ctg gat ata tat tca aga agg tta tct	caa	2571	
Gln Ala Asn Leu	Thr Glu Leu Asp Ile Tyr Ser Arg Arg	Leu Ser Gln		
	800	805	810	
gaa act ggc ttg gaa	ata agt gaa gaa att aac gaa gaa gac tta	aag	2619	
Glu Thr Gly Leu	Glu Ile Ser Glu Glu Ile Asn Glu Glu Asp	Leu Lys		
	815	820	825	
gag tgc ctt ttt gat	gat atg gag agc ata cca gca gtg act	aca tgg	2667	
Glu Cys Leu Phe	Asp Asp Met Glu Ser Ile Pro Ala Val Thr	Thr Trp		
	830	835	840	845
aac aca tac ctt cga	tat att act gtc cac aag agc tta att ttt	gtg	2715	
Asn Thr Tyr Leu	Arg Tyr Ile Thr Val His Lys Ser Leu Ile	Phe Val		
	850	855	860	
cta att tgg tgc tta	gta att ttt ctg gca gag gtg gct gct tct	ttg	2763	
Leu Ile Trp Cys	Leu Val Ile Phe Leu Ala Glu Val Ala Ala	Ser Leu		
	865	870	875	
gtt gtg ctg tgg ctc	ctt gga aac act cct ctt caa gac aaa ggg	aat	2811	
Val Val Leu Trp	Leu Leu Gly Asn Thr Pro Leu Gln Asp Lys	Gly Asn		
	880	885	890	
agt act cat agt aga	aat aac agc tat gca gtg att atc acc agc	acc	2859	
Ser Thr His Ser	Arg Asn Asn Ser Tyr Ala Val Ile Ile Thr	Ser Thr		
	895	900	905	
agt tcg tat tat gtg	ttt tac att tac gtg gga gta gcc gac act	ttg	2907	
Ser Ser Tyr Tyr	Val Phe Tyr Ile Tyr Val Gly Val Ala Asp	Thr Leu		
	910	915	920	925
ctt gct atg gga ttc	ttc aga ggt cta cca ctg gtg cat act cta	atc	2955	
Leu Ala Met Gly	Phe Phe Arg Gly Leu Pro Leu Val His Thr	Leu Ile		
	930	935	940	
aca gtg tcg aaa att	tta cac cac aaa atg tta cat tct gtt ctt	caa	3003	
Thr Val Ser Lys	Ile Leu His His Lys Met Leu His Ser	Val Leu Gln		
	945	950	955	
gca cct atg tca acc	ctc aac acg ttg aaa gca ggt ggg att ctt	aat	3051	
Ala Pro Met Ser	Thr Leu Asn Thr Leu Lys Ala Gly Gly Ile	Leu Asn		
	960	965	970	
aga ttc tcc aaa gat	ata gca att ttg gat gac ctt ctg cct ctt	acc	3099	
Arg Phe Ser Lys	Asp Ile Ala Ile Leu Asp Asp Leu Leu Pro	Leu Thr		
	975	980	985	
ata ttt gac ttc atc	cag ttg tta tta att gtg att gga gct	ata gca	3147	
Ile Phe Asp Phe	Ile Gln Leu Leu Leu Ile Val Ile Gly Ala	Ile Ala		
	990	995	1000	1005
gtt gtc gca gtt tta	caa ccc tac atc ttt gtt gca aca	gtg cca	3192	

Val Val Ala Val Leu Gln Pro Tyr Ile Phe Val Ala Thr Val Pro	
1010 1015 1020	
gtg ata gtg gct ttt att atg ttg aga gca tat ttc ctc caa acc	3237
Val Ile Val Ala Phe Ile Met Leu Arg Ala Tyr Phe Leu Gln Thr	
1025 1030 1035	
tca cag caa ctc aaa caa ctg gaa tct gaa ggc agg agt cca att	3282
Ser Gln Gln Leu Lys Gln Leu Glu Ser Glu Gly Arg Ser Pro Ile	
1040 1045 1050	
ttc act cat ctt gtt aca agc tta aaa gga cta tgg aca ctt cgt	3327
Phe Thr His Leu Val Thr Ser Leu Lys Gly Leu Trp Thr Leu Arg	
1055 1060 1065	
gcc ttc gga cgg cag cct tac ttt gaa act ctg ttc cac aaa gct	3372
Ala Phe Gly Arg Gln Pro Tyr Phe Glu Thr Leu Phe His Lys Ala	
1070 1075 1080	
ctg aat tta cat act gcc aac tgg ttc ttg tac ctg tca aca ctg	3417
Leu Asn Leu His Thr Ala Asn Trp Phe Leu Tyr Leu Ser Thr Leu	
1085 1090 1095	
cgc tgg ttc caa atg aga ata gaa atg att ttt gtc atc ttc ttc	3462
Arg Trp Phe Gln Met Arg Ile Glu Met Ile Phe Val Ile Phe Phe	
1100 1105 1110	
att gct gtt acc ttc att tcc att tta aca aca gga gaa gga gaa	3507
Ile Ala Val Thr Phe Ile Ser Ile Leu Thr Thr Gly Glu Gly Glu	
1115 1120 1125	
gga aga gtt ggt att atc ctg act tta gcc atg aat atc atg agt	3552
Gly Arg Val Gly Ile Ile Leu Thr Leu Ala Met Asn Ile Met Ser	
1130 1135 1140	
aca ttg cag tgg gct gta aac tcc agc ata gat gtg gat agc ttg	3597
Thr Leu Gln Trp Ala Val Asn Ser Ser Ile Asp Val Asp Ser Leu	
1145 1150 1155	
atg cga tct gtg agc cga gtc ttt aag ttc att gac atg cca aca	3642
Met Arg Ser Val Ser Arg Val Phe Lys Phe Ile Asp Met Pro Thr	
1160 1165 1170	
gaa ggt aaa cct acc aag tca acc aaa cca tac aag aat ggc caa	3687
Glu Gly Lys Pro Thr Lys Ser Thr Lys Pro Tyr Lys Asn Gly Gln	
1175 1180 1185	
ctc tcg aaa gtt atg att att gag aat tca cac gtg aag aaa gat	3732
Leu Ser Lys Val Met Ile Ile Glu Asn Ser His Val Lys Lys Asp	
1190 1195 1200	
gac atc tgg ccc tca ggg ggc caa atg act gtc aaa gat ctc aca	3777
Asp Ile Trp Pro Ser Gly Gly Gln Met Thr Val Lys Asp Leu Thr	
1205 1210 1215	
gca aaa tac aca gaa ggt gga aat gcc ata tta gag aac att tcc	3822
Ala Lys Tyr Thr Glu Gly Gly Asn Ala Ile Leu Glu Asn Ile Ser	
1220 1225 1230	
ttc tca ata agt cct ggc cag agg gtg ggc ctc ttg gga aga act	3867
Phe Ser Ile Ser Pro Gly Gln Arg Val Gly Leu Leu Gly Arg Thr	
1235 1240 1245	

gga tca ggg aag agt act ttg tta tca gct ttt ttg aga cta ctg Gly Ser Gly Lys Ser Thr Leu Leu Ser Ala Phe Leu Arg Leu Leu 1250 1255 1260	3912
aac act gaa gga gaa atc cag atc gat ggt gtg tct tgg gat tca Asn Thr Glu Gly Glu Ile Gln Ile Asp Gly Val Ser Trp Asp Ser 1265 1270 1275	3957
ata act ttg caa cag tgg agg aaa gcc ttt gga gtg ata cca cag Ile Thr Leu Gln Gln Trp Arg Lys Ala Phe Gly Val Ile Pro Gln 1280 1285 1290	4002
aaa gta ttt att ttt tct gga aca ttt aga aaa aac ttg gat ccc Lys Val Phe Ile Phe Ser Gly Thr Phe Arg Lys Asn Leu Asp Pro 1295 1300 1305	4047
tat gaa cag tgg agt gat caa gaa ata tgg aaa gtt gca gat gag Tyr Glu Gln Trp Ser Asp Gln Glu Ile Trp Lys Val Ala Asp Glu 1310 1315 1320	4092
gtt ggg ctc aga tct gtg ata gaa cag ttt cct ggg aag ctt gac Val Gly Leu Arg Ser Val Ile Glu Gln Phe Pro Gly Lys Leu Asp 1325 1330 1335	4137
ttt gtc ctt gtg gat ggg ggc tgt gtc cta agc cat ggc cac aag Phe Val Leu Val Asp Gly Gly Cys Val Leu Ser His Gly His Lys 1340 1345 1350	4182
cag ttg atg tgc ttg gct aga tct gtt ctc agt aag gcg aag atc Gln Leu Met Cys Leu Ala Arg Ser Val Leu Ser Lys Ala Lys Ile 1355 1360 1365	4227
ttg ctg ctt gat gaa ccc agt gct cat ttg gat cca gta aca tac Leu Leu Leu Asp Glu Pro Ser Ala His Leu Asp Pro Val Thr Tyr 1370 1375 1380	4272
caa ata att aga aga act cta aaa caa gca ttt gct gat tgc aca Gln Ile Ile Arg Arg Thr Leu Lys Gln Ala Phe Ala Asp Cys Thr 1385 1390 1395	4317
gta att ctc tgt gaa cac agg ata gaa gca atg ctg gaa tgc caa Val Ile Leu Cys Glu His Arg Ile Glu Ala Met Leu Glu Cys Gln 1400 1405 1410	4362
caa ttt ttg gtc ata gaa gag aac aaa gtg cgg cag tac gat tcc Gln Phe Leu Val Ile Glu Glu Asn Lys Val Arg Gln Tyr Asp Ser 1415 1420 1425	4407
atc cag aaa ctg ctg aac gag agg agc ctc ttc cgg caa gcc atc Ile Gln Lys Leu Leu Asn Glu Arg Ser Leu Phe Arg Gln Ala Ile 1430 1435 1440	4452
agc ccc tcc gac agg gtg aag ctc ttt ccc cac cgg aac tca agc Ser Pro Ser Asp Arg Val Lys Leu Phe Pro His Arg Asn Ser Ser 1445 1450 1455	4497
aag tgc aag tct aag ccc cag att gct gct ctg aaa gag gag aca Lys Cys Lys Ser Lys Pro Gln Ile Ala Ala Leu Lys Glu Glu Thr 1460 1465 1470	4542
gaa gaa gag gtg caa gat aca agg ctt tag agagcagcat aaatgttgac Glu Glu Glu Val Gln Asp Thr Arg Leu 1475	4592

atgggacatt tgctcatgga attggagctc gtgggacagt cacctcatgg aattggagct 4652
 cgtggaacag ttacctctgc ctcagaaaac aaggatgaat taagtttttt tttaaaaaag 4712
 aaacattttg taaggggaat tgaggacact gatatgggtc ttgataaatg gcttcctggc 4772
 aatagtcaaa ttgtgtgaaa ggtacttcaa atccttgaag atttaccact tgtgttttgc 4832
 aagccagatt ttcctgaaaa cccttgccat gtgctagtaa ttggaaaggc agctctaaat 4892
 gtcaatcagc ctagttagtc agcttattgt ctagtgaac tcgttaattt gtagtgttgg 4952
 agaagaactg aaatcatact tcttaggggt atgattaagt aatgataact ggaaacttca 5012
 gcggtttata taagcttgta ttcctttttc tctcctctcc ccatgatgtt tagaaacaca 5072
 actatattgt ttgctaagca ttccaactat ctcatttcca agcaagtatt agaataccac 5132
 aggaaccaca agactgcaca tcaaaatatg cccatttcaa catctagtga gcagtcagga 5192
 aagagaactt ccagatcctg gaaatcaggg ttagtattgt ccaggcttac caaaaatctc 5252
 aatatttcag ataatcacia tacatccctt acctgggaaa gggctgttat aatctttcac 5312
 aggggacagg atgggtccct tgatgaagaa gttgatatgc cttttcccaa ctccagaaag 5372
 tgacaagctc acagacctt gaactagagt ttagctggaa aagtatgtta gtgcaaattg 5432
 tcacaggaca gcccttcttt ccacagaagc tccaggtaga ggggtgtgta gtagataggc 5492
 catgggcact gtgggtagac acacatgaag tccaagcatt tagatgtata ggttgatggc 5552
 ggtatgtttt caggctagat gtatgtactt catgctgtct aactaagag agaatgagag 5612
 acacactgaa gaagcaccaa tcatgaatta gttttatatg cttctgtttt ataattttgt 5672
 gaagcaaat tttttctcta ggaaatatat attttaataa tgtttcaaac atatattaca 5732
 atgctgtatt ttaaaagaat gattatgaat tacatttgta taaaataatt tttatatttg 5792
 aaatattgac tttttatggc actagtattt ttatgaaata ttatgttaaa actgggacag 5852
 gggagaacct agggtgatat taaccagggg ccatgaatca ccttttggtc tggaggggag 5912
 ccttggggct gatcgagttg ttgccacag ctgtatgatt ccagccaga cacagcctct 5972
 tagatgcagt tctgaagaag atggtaccac cagtctgact gtttccatca agggtagact 6032
 gccttctcaa ctccaaactg actcttaaga agactgcatt atatttatta ctgtaagaaa 6092
 atatcacttg tcaataaaat ccatacattt gtgt 6126

<210> 62

<211> 1479

<212> PRT

<213> Cystic Fibrosis Transmembrane Conductor Regulator (CFTR) delta F508 mutation

<400> 62

Met Gln Arg Ser Pro Leu Glu Lys Ala Ser Val Val Ser Lys Leu Phe

133

1	5	10	15
Phe Ser Trp Thr Arg Pro Ile Leu Arg Lys Gly Tyr Arg Gln Arg Leu	20	25	30
Glu Leu Ser Asp Ile Tyr Gln Ile Pro Ser Val Asp Ser Ala Asp Asn	35	40	45
Leu Ser Glu Lys Leu Glu Arg Glu Trp Asp Arg Glu Leu Ala Ser Lys	50	55	60
Lys Asn Pro Lys Leu Ile Asn Ala Leu Arg Arg Cys Phe Phe Trp Arg	65	70	75
Phe Met Phe Tyr Gly Ile Phe Leu Tyr Leu Gly Glu Val Thr Lys Ala	85	90	95
Val Gln Pro Leu Leu Leu Gly Arg Ile Ile Ala Ser Tyr Asp Pro Asp	100	105	110
Asn Lys Glu Glu Arg Ser Ile Ala Ile Tyr Leu Gly Ile Gly Leu Cys	115	120	125
Leu Leu Phe Ile Val Arg Thr Leu Leu Leu His Pro Ala Ile Phe Gly	130	135	140
Leu His His Ile Gly Met Gln Met Arg Ile Ala Met Phe Ser Leu Ile	145	150	155
Tyr Lys Lys Thr Leu Lys Leu Ser Ser Arg Val Leu Asp Lys Ile Ser	165	170	175
Ile Gly Gln Leu Val Ser Leu Leu Ser Asn Asn Leu Asn Lys Phe Asp	180	185	190
Glu Gly Leu Ala Leu Ala His Phe Val Trp Ile Ala Pro Leu Gln Val	195	200	205
Ala Leu Leu Met Gly Leu Ile Trp Glu Leu Leu Gln Ala Ser Ala Phe	210	215	220
Cys Gly Leu Gly Phe Leu Ile Val Leu Ala Leu Phe Gln Ala Gly Leu	225	230	235
Gly Arg Met Met Met Lys Tyr Arg Asp Gln Arg Ala Gly Lys Ile Ser	245	250	255

Glu Arg Leu Val Ile Thr Ser Glu Met Ile Glu Asn Ile Gln Ser Val
 260 265 270

Lys Ala Tyr Cys Trp Glu Glu Ala Met Glu Lys Met Ile Glu Asn Leu
 275 280 285

Arg Gln Thr Glu Leu Lys Leu Thr Arg Lys Ala Ala Tyr Val Arg Tyr
 290 295 300

Phe Asn Ser Ser Ala Phe Phe Phe Ser Gly Phe Phe Val Val Phe Leu
 305 310 315 320

Ser Val Leu Pro Tyr Ala Leu Ile Lys Gly Ile Ile Leu Arg Lys Ile
 325 330 335

Phe Thr Thr Ile Ser Phe Cys Ile Val Leu Arg Met Ala Val Thr Arg
 340 345 350

Gln Phe Pro Trp Ala Val Gln Thr Trp Tyr Asp Ser Leu Gly Ala Ile
 355 360 365

Asn Lys Ile Gln Asp Phe Leu Gln Lys Gln Glu Tyr Lys Thr Leu Glu
 370 375 380

Tyr Asn Leu Thr Thr Thr Glu Val Val Met Glu Asn Val Thr Ala Phe
 385 390 395 400

Trp Glu Glu Gly Phe Gly Glu Leu Phe Glu Lys Ala Lys Gln Asn Asn
 405 410 415

Asn Asn Arg Lys Thr Ser Asn Gly Asp Asp Ser Leu Phe Phe Ser Asn
 420 425 430

Phe Ser Leu Leu Gly Thr Pro Val Leu Lys Asp Ile Asn Phe Lys Ile
 435 440 445

Glu Arg Gly Gln Leu Leu Ala Val Ala Gly Ser Thr Gly Ala Gly Lys
 450 455 460

Thr Ser Leu Leu Met Met Ile Met Gly Glu Leu Glu Pro Ser Glu Gly
 465 470 475 480

Lys Ile Lys His Ser Gly Arg Ile Ser Phe Cys Ser Gln Phe Ser Trp
 485 490 495

Ile Met Pro Gly Thr Ile Lys Glu Asn Ile Ile Gly Val Ser Tyr Asp
 500 505 510

135

Glu Tyr Arg Tyr Arg Ser Val Ile Lys Ala Cys Gln Leu Glu Glu Asp
 515 520 525

Ile Ser Lys Phe Ala Glu Lys Asp Asn Ile Val Leu Gly Glu Gly Gly
 530 535 540

Ile Thr Leu Ser Gly Gly Gln Arg Ala Arg Ile Ser Leu Ala Arg Ala
 545 550 555 560

Val Tyr Lys Asp Ala Asp Leu Tyr Leu Leu Asp Ser Pro Phe Gly Tyr
 565 570 575

Leu Asp Val Leu Thr Glu Lys Glu Ile Phe Glu Ser Cys Val Cys Lys
 580 585 590

Leu Met Ala Asn Lys Thr Arg Ile Leu Val Thr Ser Lys Met Glu His
 595 600 605

Leu Lys Lys Ala Asp Lys Ile Leu Ile Leu Asn Glu Gly Ser Ser Tyr
 610 615 620

Phe Tyr Gly Thr Phe Ser Glu Leu Gln Asn Leu Gln Pro Asp Phe Ser
 625 630 635 640

Ser Lys Leu Met Gly Cys Asp Ser Phe Asp Gln Phe Ser Ala Glu Arg
 645 650 655

Arg Asn Ser Ile Leu Thr Glu Thr Leu His Arg Phe Ser Leu Glu Gly
 660 665 670

Asp Ala Pro Val Ser Trp Thr Glu Thr Lys Lys Gln Ser Phe Lys Gln
 675 680 685

Thr Gly Glu Phe Gly Glu Lys Arg Lys Asn Ser Ile Leu Asn Pro Ile
 690 695 700

Asn Ser Ile Arg Lys Phe Ser Ile Val Gln Lys Thr Pro Leu Gln Met
 705 710 715 720

Asn Gly Ile Glu Glu Asp Ser Asp Glu Pro Leu Glu Arg Arg Leu Ser
 725 730 735

Leu Val Pro Asp Ser Glu Gln Gly Glu Ala Ile Leu Pro Arg Ile Ser
 740 745 750

Val Ile Ser Thr Gly Pro Thr Leu Gln Ala Arg Arg Arg Gln Ser Val
 755 760 765

Leu Asn Leu Met Thr His Ser Val Asn Gln Gly Gln Asn Ile His Arg
 770 775 780

Lys Thr Thr Ala Ser Thr Arg Lys Val Ser Leu Ala Pro Gln Ala Asn
 785 790 795 800

Leu Thr Glu Leu Asp Ile Tyr Ser Arg Arg Leu Ser Gln Glu Thr Gly
 805 810 815

Leu Glu Ile Ser Glu Glu Ile Asn Glu Glu Asp Leu Lys Glu Cys Leu
 820 825 830

Phe Asp Asp Met Glu Ser Ile Pro Ala Val Thr Thr Trp Asn Thr Tyr
 835 840 845

Leu Arg Tyr Ile Thr Val His Lys Ser Leu Ile Phe Val Leu Ile Trp
 850 855 860

Cys Leu Val Ile Phe Leu Ala Glu Val Ala Ala Ser Leu Val Val Leu
 865 870 875 880

Trp Leu Leu Gly Asn Thr Pro Leu Gln Asp Lys Gly Asn Ser Thr His
 885 890 895

Ser Arg Asn Asn Ser Tyr Ala Val Ile Ile Thr Ser Thr Ser Ser Tyr
 900 905 910

Tyr Val Phe Tyr Ile Tyr Val Gly Val Ala Asp Thr Leu Leu Ala Met
 915 920 925

Gly Phe Phe Arg Gly Leu Pro Leu Val His Thr Leu Ile Thr Val Ser
 930 935 940

Lys Ile Leu His His Lys Met Leu His Ser Val Leu Gln Ala Pro Met
 945 950 955 960

Ser Thr Leu Asn Thr Leu Lys Ala Gly Gly Ile Leu Asn Arg Phe Ser
 965 970 975

Lys Asp Ile Ala Ile Leu Asp Asp Leu Leu Pro Leu Thr Ile Phe Asp
 980 985 990

Phe Ile Gln Leu Leu Leu Ile Val Ile Gly Ala Ile Ala Val Val Ala
 995 1000 1005

Val Leu Gln Pro Tyr Ile Phe Val Ala Thr Val Pro Val Ile Val

137

1010		1015		1020
Ala Phe Ile Met Leu Arg	Ala Tyr Phe Leu Gln Thr	Ser Gln Gln		
1025	1030	1035		
Leu Lys Gln Leu Glu Ser Glu	Gly Arg Ser Pro Ile	Phe Thr His		
1040	1045	1050		
Leu Val Thr Ser Leu Lys Gly	Leu Trp Thr Leu Arg	Ala Phe Gly		
1055	1060	1065		
Arg Gln Pro Tyr Phe Glu Thr	Leu Phe His Lys Ala	Leu Asn Leu		
1070	1075	1080		
His Thr Ala Asn Trp Phe Leu	Tyr Leu Ser Thr Leu	Arg Trp Phe		
1085	1090	1095		
Gln Met Arg Ile Glu Met Ile	Phe Val Ile Phe Phe	Ile Ala Val		
1100	1105	1110		
Thr Phe Ile Ser Ile Leu Thr	Thr Gly Glu Gly Glu	Gly Arg Val		
1115	1120	1125		
Gly Ile Ile Leu Thr Leu Ala	Met Asn Ile Met Ser	Thr Leu Gln		
1130	1135	1140		
Trp Ala Val Asn Ser Ser Ile	Asp Val Asp Ser Leu	Met Arg Ser		
1145	1150	1155		
Val Ser Arg Val Phe Lys Phe	Ile Asp Met Pro Thr	Glu Gly Lys		
1160	1165	1170		
Pro Thr Lys Ser Thr Lys Pro	Tyr Lys Asn Gly Gln	Leu Ser Lys		
1175	1180	1185		
Val Met Ile Ile Glu Asn Ser	His Val Lys Lys Asp	Asp Ile Trp		
1190	1195	1200		
Pro Ser Gly Gly Gln Met Thr	Val Lys Asp Leu Thr	Ala Lys Tyr		
1205	1210	1215		
Thr Glu Gly Gly Asn Ala Ile	Leu Glu Asn Ile Ser	Phe Ser Ile		
1220	1225	1230		
Ser Pro Gly Gln Arg Val Gly	Leu Leu Gly Arg Thr	Gly Ser Gly		
1235	1240	1245		

138

Lys Ser Thr Leu Leu Ser Ala Phe Leu Arg Leu Leu Asn Thr Glu
 1250 1255 1260

Gly Glu Ile Gln Ile Asp Gly Val Ser Trp Asp Ser Ile Thr Leu
 1265 1270 1275

Gln Gln Trp Arg Lys Ala Phe Gly Val Ile Pro Gln Lys Val Phe
 1280 1285 1290

Ile Phe Ser Gly Thr Phe Arg Lys Asn Leu Asp Pro Tyr Glu Gln
 1295 1300 1305

Trp Ser Asp Gln Glu Ile Trp Lys Val Ala Asp Glu Val Gly Leu
 1310 1315 1320

Arg Ser Val Ile Glu Gln Phe Pro Gly Lys Leu Asp Phe Val Leu
 1325 1330 1335

Val Asp Gly Gly Cys Val Leu Ser His Gly His Lys Gln Leu Met
 1340 1345 1350

Cys Leu Ala Arg Ser Val Leu Ser Lys Ala Lys Ile Leu Leu Leu
 1355 1360 1365

Asp Glu Pro Ser Ala His Leu Asp Pro Val Thr Tyr Gln Ile Ile
 1370 1375 1380

Arg Arg Thr Leu Lys Gln Ala Phe Ala Asp Cys Thr Val Ile Leu
 1385 1390 1395

Cys Glu His Arg Ile Glu Ala Met Leu Glu Cys Gln Gln Phe Leu
 1400 1405 1410

Val Ile Glu Glu Asn Lys Val Arg Gln Tyr Asp Ser Ile Gln Lys
 1415 1420 1425

Leu Leu Asn Glu Arg Ser Leu Phe Arg Gln Ala Ile Ser Pro Ser
 1430 1435 1440

Asp Arg Val Lys Leu Phe Pro His Arg Asn Ser Ser Lys Cys Lys
 1445 1450 1455

Ser Lys Pro Gln Ile Ala Ala Leu Lys Glu Glu Thr Glu Glu Glu
 1460 1465 1470

Val Gln Asp Thr Arg Leu
 1475

<210> 63
<211> 36
<212> DNA
<213> synthetic oligonucleotide

<400> 63
tgagatcgat tatccttatg atgttcctga ttatgg 36

<210> 64
<211> 36
<212> DNA
<213> synthetic oligonucleotide

<400> 64
tcagcataat caggaacatc ataaggataa tcgatac 36

(19) World Intellectual Property
Organization
International Bureau



(43) International Publication Date
17 February 2005 (17.02.2005)

PCT

(10) International Publication Number
WO 2005/013666 A3

(51) International Patent Classification⁷: **G01N 33/68**

(21) International Application Number:
PCT/AU2004/001057

(22) International Filing Date: 9 August 2004 (09.08.2004)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
2003904237 8 August 2003 (08.08.2003) AU

(71) Applicant (for all designated States except US): **GARVAN INSTITUTE OF MEDICAL RESEARCH** [AU/AU]; St Vincent's Hospital, 384 Victoria Street, Darlinghurst, New South Wales 2010 (AU).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **JAMES, David** [AU/AU]; 25 Cutler Road, Clontarf, New South Wales 2093 (AU). **GOVERS, Roland** [NL/NL]; Vioolstraat 25, NL-4702 CK Roosendaal (NL).

(74) Agent: **F. B. RICE & CO**; 605 Darling Street, Balmain, NSW 2041 (AU).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM,

AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

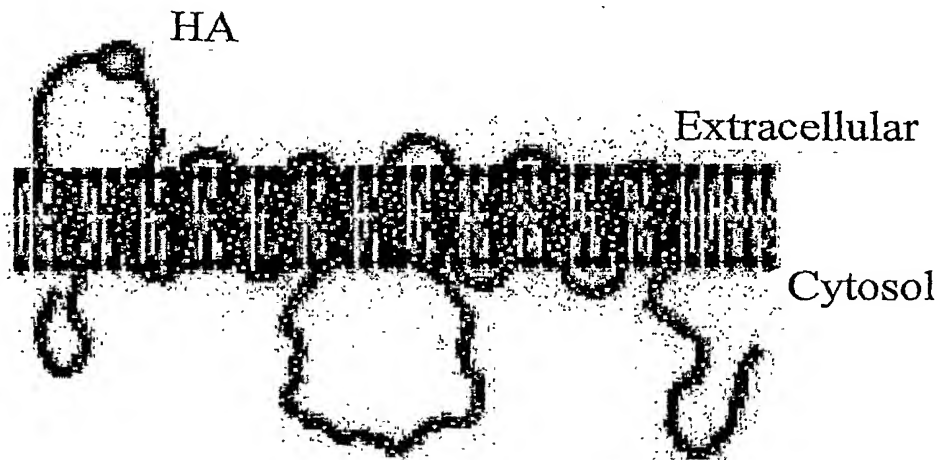
Published:

- with international search report
- before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments

(88) Date of publication of the international search report:
28 April 2005

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: NOVEL TRANSLOCATION ASSAY



(57) Abstract: The present invention relates to a novel in vitro assay for determining the level of a protein, in particular, a membrane transport protein that is located at the plasma membrane of a cell compared to the level of the protein in the cell. The process of the invention is also useful for determining the level of recycling of a membrane transport protein. The present invention additionally provides a process for identifying an agent that modulates the translocation of a protein, in particular, a membrane transport protein, to the plasma membrane and, as a consequence, the activity of that protein.

WO 2005/013666 A3

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU2004/001057

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl. ⁷: G01N 33/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See "electronic data base" box below

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

See "electronic data base" box below

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

MEDLINE, CAPLUS, WPIDS: glut, glut1, glut4, traffic?, translocat?, tag, marker, ligand, bind, bound, receptor, lys?, disrupt?, permeabilis?, rupture?, assay, process, method, level

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 6 303 373 B1 (BOGAN et al.) 16 October 2001 Whole specification	1 (in part), 2-62
P,A	US 6 632 924 B2 (BOGAN et al.) 14 October 2003 Whole specification	1 (in part), 2-62
A	US 5 989 893 A (CZECH et al.) 23 November 1999 Whole specification	1 (in part), 2-62
A	SLOT, J.W. et al., "Translocation of the glucose transporter GLUT4 in cardiac myocytes of the rat", Proc. Natl. Acad. Sci. USA, September 1991, Vol. 88, pages 7815-7819 Whole article	1 (in part), 2-62



Further documents are listed in the continuation of Box C



See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"T"

later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"E" earlier application or patent but published on or after the international filing date

"X"

document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"Y"

document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"O" document referring to an oral disclosure, use, exhibition or other means

"&"

document member of the same patent family

"P" document published prior to the international filing date but later than the priority date claimed

Date of the actual completion of the international search
7 February 2005

Date of mailing of the international search report
21 FEB 2005

Name and mailing address of the ISA/AU
AUSTRALIAN PATENT OFFICE
PO BOX 200, WODEN ACT 2606, AUSTRALIA
E-mail address: pct@ipaustalia.gov.au
Facsimile No. (02) 6285 3929

Authorized officer

JAMIE TURNER

Telephone No : (02) 6283 2071

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU2004/001057

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	HANEY, P.M., "Intracellular Targetting of the Insulin-regulatable Glucose Transporter (GLUT4) Is Isoform Specific and Independent of Cell Type", The Journal of Cell Biology, August 1991, Vol. 114, No. 4, pages 689-699 Whole article	1 (in part), 2-62
X	WANG et al., "GLUT4 Translocation by Insulin in Intact Muscle Cells: Detection by a fast and Quantitative Assay", FEBS Letters, 1998, Vol. 427, pages 193-197 See especially paragraph 3.4 at page 195	1 (in part), 2-62

INTERNATIONAL SEARCH REPORT

International application No.

PCT/AU2004/001057

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 1
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
Claim 1 is not limited to the technical features of the invention described in the international application. Clearly, there is support only for the determination of membrane transport proteins which are glucose transport (GLUT) proteins, notably GLUT1 and GLUT4. Because claim 1 is not limited to the detection of GLUT proteins it is not considered limited to the technical features of the invention.
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a)

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU2004/001057

Supplemental Box

(To be used when the space in any of Boxes I to VIII is not sufficient)

Continuation of Box No:

It should be noted that an amended page 108, containing amendments to claims 53 and 54, was filed 5 October 2004 with the International Bureau. Unfortunately, because these amendments were not filed in the time frame referred to in Article 19 and Rule 46.1, these amendments cannot be considered under Art. 19. However, it should be noted that the subject matter of claims 53-54 (as if they were amended) was nevertheless the subject of the International Search Report. Further, it is apparent that the amendments to these pages do not go beyond the disclosure of the international application as filed.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/AU2004/001057

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report				Patent Family Member			
US	6303373	AU	54775/00	EP	1189943	US	6632924
		US	2002052012	US	2002155479	WO	0075188
		WO	02059299				
US	5989893	AU	78448/94	EP	0721508	WO	9509240
Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.							
END OF ANNEX							